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COPYRIGHT, 1944, BY THE AMERICAN MEDICAL ASSOCIATION

MECHANISM OF THE ISOIMMUNIZATION BY THE RH FACTOR OF RED BLOOD CELLS

STANDARDIZATION OF ANTI-RH SERUMS

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NEWARK, N. J.

It is now established that the phenomenon of isoimmunization by the Rh factor of red blood cells is of considerable importance in clinical medicine. More specifically, the Rh factor is responsible for at least 90 per cent of the intragroup transfusion reactions occurring either at the first transfusion following pregnancy or after repeated transfusion. Isoimmunization of the mother whose red blood cells do not contain the Rh factor by the blood of a fetus whose red cells contain it has been shown to be the basis for the genesis of erythroblastosis fetalis.[1] It is obvious that the Rh factor is especially important in civilian practice. However, it is also of considerable interest to the medical services of the armed forces because a certain proportion of wounded persons will require repeated transfusions. Moreover, it must be kept in mind that some of the hospitals under military control provide medical care also for the wives and families of the officers and enlisted men.

The Rh factor is a good antigen in man, so that a patient with Rh-negative blood receiving repeated transfusions of Rh-positive blood or a mother with Rh-negative blood bearing a fetus with Rh-positive blood sooner or later becomes immunized.[2] In the former case a particular transfusion may be followed by a mild reaction while the following transfusions will result in increasingly severe reactions, which may end in fatal anuria. In the group of women immunized by Rh-positive fetal blood, however, it is characteristic that the very first transfusion may terminate in severe or even fatal anuria.

The intrauterine hemolysis which is so characteristic of the clinical and pathologic picture of erythroblastosis fetalis obviously derives from the action of the maternal anti-Rh agglutinins on the susceptible Rh-containing fetal red blood cells.

The capacity to produce anti-Rh agglutinins differs in individual subjects so that in some women the first pregnancy may result in an erythroblastotic infant while in other women whose blood is Rh negative such infants are not borne until there have been several pregnancies with normal infants whose blood cells contain the Rh antigen. When the antigenic stimulus is derived from repeated blood transfusions some patients with Rh-negative blood acquire intolerance to Rh-positive blood after a few uneventful transfusions, while others will tolerate numerous transfusions of Rh-positive blood before there is evidence of isoimmunization. It is likely that the capacity for isoimmunization in general depends on one or more genetic properties. In any event, pregnancy offers conditions more suitable for isoimmunization than repeated transfusions, and the main factor probably is the slow administration of the antigen over a long period.[3]

In 1939 Levine and Stetson[4] ascribed the origin of an atypical agglutinin produced by a mother who harbored a macerated fetus, to isoimmunization by a hitherto unknown factor in the red blood cells of the fetus. They stressed the transplacental origin of the isoimmunization. However, it was generally overlooked in the literature that Levine and Stetson actually described a new agglutinable blood factor, independent of A, B, M, N or P, which could be demonstrated by means of this isoagglutinin. This new factor was found to be present in about 80 per cent of all bloods.

From the Division of Laboratories, Newark Beth Israel Hospital.

This study was aided by grants from the National Committee on Maternal Health and the Blood Transfusion Association of New York.

1. (*a*) Levine, P.; Burnham, L.; Katzin, E. M., and Vogel, P.: Am. J. Obst. & Gynec. **42**:925, 1941. (*b*) Levine, P.: New York State J. Med. **42**:1928, 1942.

2. (*a*) Wiener, A. S.: Arch. Path. **32**:227, 1941. (*b*) Levine, P.: Am. J. Clin. Path. **11**:898, 1941. (*c*) Diamond, L. K.: New England J. Med. **227**:857 and 932, 1942. (*d*) Vogel, P.; Rosenthal, N., and Levine, P.: Am. J. Clin. Path. **13**:1, 1943. (*e*) Boorman, K. E.; Dodd, B. E., and Mollinson, P. L.: Brit. M. J. **2**: 535 and 569, 1942. Levine and others.[1a] Levine.[1b]

3. Levine, P.: J. Hered. **34**:71, 1943.

4. Levine, P., and Stetson, R.: J. A. M. A. **113**:126, 1939.

Soon after the genesis of erythroblastosis fetalis was described and potent human anti-Rh agglutinins became available (December 1940), it was observed by Levine and Katzin that this patient's blood was negative with anti-Rh serum and that her agglutinins were anti-Rh in specificity.[5] Accordingly, it may be stated that Landsteiner and Wiener[6] in 1940 observed an agglutinable factor in the blood of rhesus monkeys related to the factor in human blood previously described by Levine and Stetson. It is also significant that all subsequent observations which led finally to the description of the genesis of erythroblastosis fetalis were made exclusively with anti-Rh serums produced by mothers of affected infants. In contrast to the reactions of the experimental serum of Landsteiner and Wiener,[6] which are read by the quality of the sediment, human anti-Rh serums give distinct and well defined agglutination, so that there has been no need for the use of the experimental serum.

Considerable information is now available for the prevention of intragroup transfusion reactions and for the treatment of erythroblastotic infants. In brief, all patients whose blood is Rh-negative and the affected infants, whose blood is Rh-positive, require transfusion of Rh-negative blood. Accordingly, the selection of persons with respect to Rh-positive and Rh-negative blood is of paramount clinical importance. At the present time the reagent of choice is the anti-Rh serum obtained either from patients immunized by repeated transfusions or, more frequently, from mothers immunized by the blood of erythroblastotic infants. However, since the Rh factor is a complex antigen, several varieties of anti-Rh agglutinins have been described, some of which are more suitable than others for diagnostic purposes. The present paper deals mainly with the mechanism of transplacental isoimmunization, the varieties of human anti-Rh serum and the criteria for the standardization of these diagnostic reagents. Three clinical cases which have a bearing on theoretic and practical aspects of isoimmunization are also included.

THE ANAMNESTIC REACTION

Once a person whose blood is Rh-negative is immunized either by Rh-positive fetal blood or by repeated transfusions, that person remains immunized for many years if not permanently. One case is reported here in which a transfusion of Rh-positive blood carried out eleven years after the last antigenic stimulus was tolerated, but the following transfusion of blood from the same donor resulted in severe shock and considerable damage to the kidneys. Presumably this case presents a typical example of the phenomenon known as the anamnestic reaction. This term, well known by immunologists, is defined as the "production in response to an antigenic stimulus of an antibody that has been produced in the tissues on some previous occasion.[7]" Obviously, the first transfusion serves to reawaken the antibody-producing mechanism which was stimulated during pregnancies by Rh-positive fetal blood. The long duration of isoimmunization is evident from 2 additional cases. Brief histories of these 3 cases are now presented.[8]

CASE 1.—Mrs. F. R. (blood group 0) was admitted in May 1940 to one of the metropolitan hospitals in New York because of an orthopedic condition. At this time she was treated uneventfully by transfusion of blood from her oldest son (blood group 0), then 22 years of age. In January 1941 she was given a second transfusion of blood from the same donor, but this transfusion was followed by a severe reaction with anuria from which she recovered. This patient's blood was later found to be Rh negative, and weak anti-Rh agglutinins were present. Her son's blood was Rh positive. It is clear that in this patient isoimmunization could not be produced by only one transfusion. However, the first transfusion reactivated the antibody-producing mechanism which had been stimulated previously during pregnancies with fetuses whose blood was Rh positive. The obstetric history of this patient is significant. In all there were six pregnancies, two resulting in normal children, three in spontaneous abortions and the last pregnancy, eleven years previously, in a jaundiced infant who required transfusions.

CASE 2.—Mrs. J. B. (blood group A), aged 56 at the time of writing, had a daughter about 37 years old; there had been no other pregnancies. In 1937 she had one uneventful transfusion of Rh-positive blood from her daughter (blood group A), given because of mastoiditis. In May 1941 a diagnosis of leukemia was made. A transfusion from the same donor given at this time resulted in a delayed reaction. The next transfusion from another donor (blood group A, Rh-positive blood), given Aug. 7, 1941, resulted in a violent reaction, followed by oliguria. Subsequently, she was uneventfully treated with a transfusion of Rh-negative blood. As in the previous case, the patient's blood was Rh negative and the blood of each donor was Rh positive. Although there is no history of an erythroblastotic infant, it is probable that the one pregnancy thirty-seven years previously (1904) initiated the isoimmunization. There was an additional stimulus resulting from the uneventful transfusion years later (1937), so that the transfusion given in May 1941 resulted in a slight reaction.

CASE 3.—Mrs. W. (blood group AB), 54 years old, was suffering from carcinoma of the colon. She had 6 healthy children, the youngest of whom at the time of writing was 17. The fifth child, 22 at the time of writing, was jaundiced at or shortly after birth. In 1932 the patient had dilation and curettage of the uterus for excessive menopausal bleeding. At this time she was given a transfusion, in the course of which she had a pain in the midback. Shortly thereafter she was extremely jaundiced. During an illness in April 1943

5. These tests are mentioned in a previous paper.[1a]

6. Landsteiner, K., and Wiener, A. S.: Proc. Soc. Exper. Biol. & Med. **43**:223, 1940.

7. Topley, W. W. C.: An Outline of Immunity, Baltimore, William Wood & Company, 1933, p. 183.

8. Case 1, studied by Levine and Katzin, has been described elsewhere (Katzin, E. M.: Mississippi Valley M. J. **64**:171, 1942). Case 2 was referred by Drs. J. Gray and W. Bernhard. The pertinent information in case 3 was supplied by Dr. J. J. McGraw. The Rh studies in this case made by Dr. McGraw were confirmed.

and prior to an operation she was given a transfusion and suffered a severe chill. (Pyrogenic reactions at this hospital are exceedingly rare.) One week later it was found that her blood was Rh negative and that her serum contained anti-Rh agglutinins. She was then successfully given a transfusion of Rh-negative blood.

From a practical standpoint all patients with Rh-negative blood who require transfusions should always receive Rh-negative blood, and I have made it a policy to inform all such patients of this precaution. More specifically, a history of repeated transfusions, especially if these have been accompanied with reactions, is important even if the patient, male or female, is to be given a transfusion many years later. Similarly, an obstetric history suggestive of isoimmunization (fetal or neonatal morbidity) may be significant even if the patient is to be given a transfusion considerably after her child-bearing age. These considerations may apply also for those women with Rh-negative blood who have one or two normal children with Rh-positive blood but who nevertheless may be immunized at least to a slight degree, as is indicated in case 2.

It is clear that the anamnestic reaction now becomes important clinically, as cases of this sort must occur frequently. A review of my material reveals an additional 2 instances, and Weiss[9] recently observed this phenomenon in 3 patients. Although the concept of isoimmunization is of particular interest to specialists in several branches of medicine, the occurrence of cases similar to those described makes it necessary for all physicians to appreciate the various manifestations of isoimmunization.

The anamnestic reaction is significant also theoretically in connection with the mechanism of the isoimmunization of the mother whose blood is Rh negative by Rh-positive fetal blood.

MECHANISM OF THE ISOIMMUNIZATION IN PREGNANCY

Since the Rh factor is limited to red blood cells, the antigenic stimulus in a mother whose blood is Rh negative must be derived from the Rh-containing fetal blood cells.[10] The indications are that in every normal pregnancy minute quantities of fetal blood may find their way into the maternal circulation by virtue of microscopic breaks in the fetal villi. It does not seem necessary to assume the presence of gross lesions in the placenta. In this connection it may be pertinent to mention an experiment in which rabbits were immunized with daily intravenous injections of minute quantities of human blood. After seven injections of 2 cc. of a 1:5,000 suspension of citrated blood there was a distinct increase in the titers varying from 1:25 to 1:600; furthermore, in 1 of the 5 rabbits weak anti-M agglutinins developed. The details of this experiment are given in table 1.

Measured in terms of sediment, the total volumes of blood employed for the seven and the fourteen injections (table 1) were respectively 0.0014 and 0.0028 cc. The corresponding values for an adult of 120 pounds (60 Kg.) are 0.0336 and 0.0672 cc. respectively. In view of the enormous surface of fetal villi exposed to the maternal sinuses it is conceivable that small quantities of fetal blood, sufficient for isoimmunization, may be readily available even under the physiologic conditions of pregnancy. According to Dodds,[11] the total area of villi of a full term placenta exposed to the maternal stream is 70 sq. ft. If the diameter of a free absorbing villus is assumed to be from 0.03 to 0.06 mm., the total length of the villi if they were placed end to end would be 11.4 miles (18.24 kilometers). The same author stated that one fourth or more of the fetal blood is outside the fetus and in the placenta. In this connection it is significant that

TABLE 1.—*Immunization of Rabbits with Minute Quantities of Human Blood (Group 0)*

	Agglutination Titers of Five Immunized Rabbit Bloods Tested with Blood Group O				
Time of Test	1	2	3	4	5
Before injections	5	10	5	5	5
After 7 injections	15	100	25	25	10
After 14 injections	100	300	25	600	100

the course of the pregnancy and the delivery of the erythroblastotic infant are, as a rule, normal. Toxic symptoms are apt to occur only in some of the much smaller group of mothers of infants with fetal hydrops, and it is probable that these symptoms cannot be attributed to the effects of gross leakage.[12]

9. Weiss, C.: Oral communication to the author.

10. Levine, P., and Katzin, E. M.: Proc. Soc. Exper. Biol. & Med. **48**:126, 1941. Levine and others.[1a]

11. Dodds, G. S.: Anat. Rec. **24**:287, 1922-1923.

12. I recently discussed the mechanism of isoimmunization with Dr. A. T. Hertig, who on request kindly submitted the following statement. "The possibility of pathological leakage of minute quantities of fetal blood has also been suggested by Hertig who, in keeping with others, e. g. Schmorl, has seen detached fragments of syncytiotrophoblast in a high percentage of the lungs of patients dying late in pregnancy of whatever cause. It is possible, therefore, that the breaking off of such fragments into the maternal blood stream is accompanied by a loss of continuity of the surface of the villus which allows the escape of minute amounts of fetal blood into the maternal circulation. Furthermore, it is theoretically possible that occasional small terminal branches of chorionic villi are broken off, since Hertig has observed that such portions of villi may prolapse into the venous endometrial sinusoids of the placental floor. With the physiologic contractions of the uterus,

(Footnote continued on next page)

The mechanism of isoimmunization described in the foregoing paragraph is compatible with the clinical observation that once a mother with Rh-negative blood delivers an erythroblastotic infant, the condition is apt to recur in all succeeding pregnancies with fetuses whose red cells contain the Rh factor. Apparently the isoimmunization is renewed even if subsequent pregnancies are spaced at long intervals. In a sense, then, the anamnestic reaction is called into play in these subsequent pregnancies. Whether or not the isoimmunization can be prevented by blocking or inducing fatigue in the reticuloendothelial system remains to be seen.

Isoimmunization against the Rh factor of fetal red blood cells depends on (1) a combination of a mother whose blood is Rh negative and a fetus whose red cells contain the Rh factor, i. e., one or more pregnancies with fetuses whose red cells contain the Rh factor and (2) genetic capacity to respond to the antigenic stimulus. It is assumed that the mother whose first infant with Rh positive blood is born with erythroblastosis fetalis produces antibodies with great ease in contrast to the mother who has several normal children with Rh-positive blood before an affected infant is born. The two factors mentioned serve to determine the incidence of erythroblastosis fetalis, which is comparatively low in spite of the fact that in 13 per cent of all matings in a random white population the father has the Rh factor in his erythrocytes (85 per cent) and the mother does not have it (15 per cent). Racial differences in the incidence of erythroblastosis fetalis in white, Negro and Chinese races were published elsewhere.[13]

Assuming that minute quantities of fetal blood find their way into the maternal circulation in normal pregnancy, one need not be surprised that other factors which may be present in fetal blood (A. B. Hr) are also capable of inducing isoimmunization with resulting damage to the fetus.[14] Erythroblastosis fetalis becomes important altogether out of proportion to its comparatively low incidence because it establishes a precedent for fetal and neonatal morbidity attributable to a hereditary antigenic difference in the blood of man and wife. In this connection it is significant that the cause of a large proportion of miscarriages and a smaller proportion of stillbirths is still a mystery.

SPECIFICITY OF HUMAN ANTI-RH SERUMS

The early studies on the specificity of human anti-Rh serums revealed the presence of at least three varieties of agglutinins.[15] Actually these three varieties (tentatively termed anti-Rh_1, anti-Rh_2, and anti-$Rh_{1, 2}$) result from the varying combinations of two qualitatively different agglutinins.[13a] The evidence supporting these facts was derived by Levine and co-workers from parallel tests on 334 random bloods of all groups with these three kinds of anti-Rh serums. These tests could be carried out because large quantities of potent anti-Rh serums were available soon after the genesis of erythroblastosis fetalis was described. The anti-Rh serums were selected from numerous mothers of erythroblastotic infants referred by physicians in the United States and Canada. As an essential preliminary, it was necessary to establish the relationship of these serums to each other in tests on random bloods before undertaking a statistical study of a series of erythroblastotic infants.

TABLE 2.—*Agglutination Reactions of Three Anti-Rh Serums with Three Hundred and Thirty-Four Random Bloods of All Groups*

(The serums were obtained from mothers of erythroblastotic infants. These tests were carried out in April, May and June of 1941)

Rh Blood Type According to Wiener and Landsteiner	Incidence of Type, Percentage	Reaction with Given Serum		
		Mrs. M. F. anti-Rh	Mrs. M. S. anti-Rh_1	Mrs. E. B. anti-Rh′
Rh_1	71	+	+	+
Rh_2	14	+	—	+
Rh′	2	—	+	+
Rh⁻	13	—	—	—
Incidence of + reactions.....		85%	73%	87%
Incidence of — reactions.....		15%	27%	13%

The four varieties of bloods resulting from the varying agglutination reactions are given in table 2 with the incidence of each type. The terms proposed recently by Wiener and Landsteiner [16] on the basis of their genetic studies are also included.

It will be observed that Rh′ and Rh_2 bloods are cross specific. This observation and the fact that Rh′ bloods gave weak reactions with anti-Rh′ serums led Levine [13a] to carry out absorption tests which indicated that the anti-Rh′ serum contains a mixture of anti-Rh and anti-Rh_1 agglutinins.

especially in the last trimester of pregnancy, a theoretic possibility exists whereby such prolapsed villous branches could be mechanically broken off and thus float free in the maternal circulation. Accompanying this phenomenon, there would undoubtedly be more or less fetal hemorrhage from the torn ends of the parent villus. If such a phenomenon did occur (and it has never actually been demonstrated) a mechanism would exist for the immunization of the mother whose blood is Rh negative by the Rh-containing fetal red cells."

13. (*a*) Levine, P.: Science **96**:452, 1942. (*b*) Levine, P., and Wong, H.: Am. J. Obst. & Gynec. **45**:832, 1943.

14. Levine, P.: West. J. Surg. **50**:468, 1942; footnote 3.

15. (*a*) Landsteiner, K., and Wiener, A. S.: J. Exper. Med. **74**:309, 1941. (*b*) Levine and others.[13a] Wiener.[2a] Levine.[13a]

16. Wiener, A. S., and Landsteiner, K.: Proc. Soc. Exper. Biol. & Med. **53**:167, 1943.

From the practical standpoint of the diagnosis of erythroblastosis fetalis and the prevention of intragroup transfusion accidents the anti-Rh serum is the serum of choice. Of the remaining two, anti-Rh′ serum is more important than anti-Rh_1 serum. These facts emerge from a finer analysis of the data on 153 mothers of erythroblastotic infants, previously published.[18a] At that time the results were presented in summary form so that the varying importance of the three types of serum was not immediately evident. The revised data based on a small number of additional tests with two of the serums are given in table 3.

In the case of the anti-Rh_1 serum the figures previously reported included as having Rh-negative blood 4 mothers who would have to be omitted because the bloods of their husbands or of their affected infants contained the Rh_2 factor. These mothers could be excluded because of lack

TABLE 3.—*Studies with Bloods from Selected Mothers* *

	Percentage +	Percentage −
Anti-Rh serum (Mrs. M. F.)		
334 random bloods	85	15
142 bloods from selected mothers †	10	90
Anti-Rh_1 serum (Mrs. M. S.)		
334 random bloods	73	27
28 bloods from selected mothers †	7	93
39 bloods from selected mothers §	23	77
Anti-Rh′ serum (Mrs. E. B.)		
334 random bloods	87	13
60 bloods from selected mothers †	15	85
64 bloods from selected mothers §	19	81

* The mothers were selected because they had delivered one or more infants whose condition was diagnosed as erythroblastosis fetalis.
† These figures have been published elsewhere (Levine [18a]).
§ The figures have been corrected on the basis of additional tests.

of evidence of isoimmunization as indicated by this particular serum. The corrected figures include several mothers whose red blood cells contained the rare factor Rh′. Two of the latter group are now included also in the tests with anti-Rh′ serums, and the third Rh′ mother was mentioned previously.

In contrast to the results previously presented, the corrected data permit an evaluation of the three sorts of serums. The reagent of choice, as previously stated, is obviously the anti-Rh serum. Of the two remaining sorts the anti-Rh′ serum is far more important than the anti-Rh_1 serum and actually is only slightly less valuable than the anti-Rh serum.

In employing exclusively anti-Rh serum the only cases of isoimmunization which cannot be detected are those in which the father's erythrocytes contain the factor Rh′. On the basis of the data presented in table 3, this occurs in 0.26 per cent of all matings (2 per cent × 13 per cent), while the corresponding value based exclusively on tests with anti-Rh′ serum is 1.7 per cent (85 per cent × 2 per cent). Of the 84 mothers whose blood was negative with anti-Rh serum and not tested with the other serums it can be calculated that all but 2 would also have negative results if their blood were tested with anti-Rh′ serum. Under these conditions the incidence of positive and negative reactions in tests of the blood of the selected mothers would not vary much whether the tests were made with anti-Rh or with anti-Rh′ serums.

It is true that the blood of 82 of these 84 mothers will also be negative with anti-Rh_1 serum. However, the limited value of this serum as compared with anti-Rh′ serum could be established more definitely in an analysis of the incidence of matings because in a certain number of instances the father's red blood cell will contain the factor Rh_2 and thus fail to react with anti-Rh_1 serum. A statistical analysis of this sort to be accurate should be based on a study of the bloods of the father, the mother and the affected infant. This cannot be carried out until the genetics of the various antigenic components of the Rh factor is more thoroughly understood.[17]

For the past two years, a specific difference of the bloods of the father and the mother if the latter's erythrocytes contain Rh_2 has not been accepted as evidence of isoimmunization. This is based on the data presented and also on the fact that under these conditions anti-Rh agglutinins have never been observed. In other words, the person whose red blood cells contain the Rh_2 factor cannot be immunized by other components of the Rh antigen.[17a] This is of theoretic interest because isoimmunization has been demonstrated in persons who possess the Rh′ factor.[18]

Conclusive support for the recommendation that the anti-Rh_1 serum should not be used by itself as a diagnostic reagent is derived from racial studies with the three varieties of anti-Rh serum and the incidence of erythroblastosis fetalis. Tests with anti-Rh (or anti-Rh′) serum show that erythroblastosis fetalis is at least twice as frequent in the white as in the Negro race; the latter serum gives an incidence of only 5 to 8 per cent negative reactions instead of 15 per cent (or 13 per cent) for the white race.[18] But with anti-Rh_1 serum negative reactions are twice as frequent in bloods from Negroes as in

17. (*a*) Wiener, A. S.: Science **98**:182, 1943. (*b*) Wiener and Landsteiner.[16]

17a. While this paper was in press, one such case was observed by Waller and Levine. A report is in preparation.

18. Landsteiner and Wiener.[15] Levine.[18a]

those from white persons (54 per cent and 27 per cent, respectively).[18a]

In practice I use routinely anti-Rh serum and in addition anti-Rh_1 serum in order to detect instances of isoimmunization attributable to the Rh′ factor. Anti-Rh_1 serum is actually preferable to the clinically more important anti-Rh′ serum. In the first place it is necessary to reserve for the sake of economy the supply of anti-Rh′ serum. Then, in certain cases of isoimmunization in which the patient produces only anti-Rh_1 agglutinins it should be possible to select compatible donors from the group of 27 per cent whose bloods fail to react with anti-Rh_1 serum, instead of from the small group of 13 per cent whose blood is Rh negative.[19] This is a matter of considerable importance since in practice the list of available donors of Rh-negative blood has frequently been exhausted because of a tendency to employ them indiscriminately.[20]

From an analysis of the antigenic components of the Rh factor as revealed in table 1 it is clear that the váriety of anti-Rh agglutinins produced depends on the Rh components of the mother's and the affected infant's erythrocytes. Thus in the case of the father's and the affected child's blood reacting with all anti-Rh serums, the mother's serum may contain either one of the two or, if none of the antigenic components are present in her cells, both antibodies. However, if the mother's blood is of the rare variety (2 per cent), the only agglutinin to be produced will be anti-Rh. A mother of this type (a patient of Dr. L. Burnham) was observed early in the course of these studies.[18a] Additional evidence to support the view presented has recently been provided by Wiener.[21]

In tests of several hundred bloods with the three varieties of anti-Rh serums an occasional blood will be found which does not conform to one of the four types listed in table 2. A blood of this sort was described previously.[22] In another instance, evidence of isoimmunization in a mother of an erythroblastotic infant could be obtained with only one of two anti-Rh′ serums. These observations and in general the findings with isoimmune serums in animals indicate a still further complexity of the Rh antigen. Additional evidence was recently provided by Wiener,[17a] who described still another anti-Rh agglutinin, identified as anti-Rh_2, which acts on 30 per cent of all bloods.

RH-POSITIVE BLOOD IN MOTHERS OF ERYTHROBLASTOTIC INFANTS

As shown in table 3, at least 90 per cent of all cases of erythroblastosis fetalis can be attributed to isoimmunization by the common variants of the Rh factor, i. e., Rh_1, Rh_2 and Rh′. The value may be still higher because in the remaining 10 per cent it seemed necessary to include several cases in which the diagnosis was not thoroughly established.[23] At any rate, it is possible that a systematic study of the exceptional cases with numerous anti-Rh serums will occasionally yield evidence of isoimmunization on the basis of the finer differences of the Rh factor as mentioned in a foregoing paragraph.

In the larger part of the exceptional 10 per cent of mothers whose blood is Rh-positive and who have given birth to erythroblastotic infants the isoimmunization was induced by still other factors of human blood, such as A, B, Hr and perhaps still others. That the A and B factors are antigenic in man has been shown by Jonsson,[24] Levine [25] and others. I have shown that in this group of mothers there is a higher incidence of matings with men of incompatible blood than in the series of mothers whose blood is Rh negative.

In the case of 1 mother in the series with Rh-positive blood presented in table 3 an atypical agglutinin was observed which acted distinctly on all Rh-negative and Rh_2-positive bloods and more weakly on only some of the bloods containing factors Rh_1 and Rh′. In this case the blood of the father and that of the affected infant were Rh negative. Nevertheless, the criteria of isoimmunization are satisfied with the demonstration of an atypical agglutinin which acted on the blood of the husband and on that of the affected infant.[26] The letters Hr (the reverse

19. A similar idea was recently expressed by L. K. Diamond (oral communication). Before resorting to this procedure, however, one must establish the absence of very weak anti-Rh agglutinins. Furthermore, it is now recognized that transfusion reactions may be attributable to the action of anti-Rh antibodies fixed to the tissue cells.[2a, b]

20. Obstetricians and other physicians should be warned not to use Rh-negative donated blood in giving transfusions to a patient without tests to establish that the patient's blood is Rh negative.

21. Wiener, A. S.: Oral communication to the author.

22. Levine, P.; Katzin, E. M.; Vogel, P., and Burnham, L., in Mudd, S., and Thalhimer, W.: Blood Substitutes and Blood Transfusion, Springfield, Ill., Charles C Thomas, Publisher, 1942.

23. The clinical criteria for the diagnosis of erythroblastosis fetalis should be more rigid in regard to the group of mothers with Rh-positive blood.

24. Jonsson, B.: Acta path. et microbiol. Scandinav. **13**:424, 1936.

25. Levine, P.: Unpublished data.

26. References to this patient of Dr. Javert will be found in other articles (Javert, C.: Am. J. Obst. & Gynec. **43**:921, 1942. Levine and others.[1a] Levine.[1b] Levine [3]).

of Rh) and anti-Hr were chosen to designate the new agglutinable factor and its antibody in order to indicate its reciprocal relationship to the Rh factor.

A second anti-Hr serum was recently observed in a mother with Rh-positive blood who had borne an erythroblastotic infant.[27]

STANDARDIZATION OF HUMAN ANTI-RH SERUMS

At present human anti-Rh serums seem preferable to the experimental serum produced by injection of rhesus or human blood.[28] The main advantage is the greater ease of differentiating positive and negative reactions by agglutination than by the appearance of the sedimented cells.

As already indicated, anti-Rh′ or anti-Rh (but not anti-Rh_1) serums are satisfactory for clinical purposes. Accordingly, human anti-Rh serums must be standardized not only for potency but also for specificity. Obviously, unless the serums have high or moderately high titers, it may be difficult to determine the specificity. Accordingly, a large number of anti-Rh serums should be available for selection of the most potent ones. It is difficult to give an absolute titer value for a satisfactory serum. Theoretically, it seems desirable to adopt the same criteria as for the selection of potent grouping serums, anti-A and anti-B. In view of the limited supply of human anti-Rh serums, it may be advisable, tentatively at least, not to use anti-Rh serums unless they have a titer of 1:20 in tests carried out at 37 C.

The specificity of anti-Rh serums may readily be determined in tests with a small number of bloods of known Rh antigenic composition as indicated in table 2. Because Rh′ bloods are rare, it may not be possible to differentiate immediately anti-Rh′ from anti-Rh serums. This, however, can readily be done by absorption experiments with known bloods, which may or may not reveal the presence of both antibodies, anti-Rh and anti-Rh_1, in a particular serum. Without resorting to specific absorption, titration with selected bloods may give significant results in certain cases. Thus, some anti-Rh′ serums were found to contain a potent anti-Rh_1 agglutinin but a weaker anti-Rh agglutinin. A serum exhibiting this property is not a useful diagnostic reagent unless the weaker agglutinin has a titer of about 1:20.

Two other criteria of human anti-Rh serums to be satisfied are the ready inhibition of the normal isoantibodies by soluble A and B substances of Witebsky (or by saliva)[29] and the absence of the so-called "zone effect." In some anti-Rh serums the normal isoantibodies, particularly anti-B, are inhibited with great difficulty. Apparently this may be due to a special property of the isoantibody since the effect seems to be independent of the titer. Such serums require considerably larger quantities of the group-specific substances for the complete inhibition of these antibodies. For the vast majority of anti-Rh serums, 1 volume of the A and B inhibiting material mixed with 4 volumes of serum suffices, but some anti-Rh serums were found to require twice or even eight times the usual amounts of the group-specific preparations (table 4).

In most instances such serums may be used if sufficient inhibiting substances are added. This can be done safely since the A and B inhibiting substances do not specifically inhibit the anti-Rh

TABLE 4.—*Inhibition of Normal Isoagglutinin Anti-B*

Each anti-Rh serum (0.5 cc.) was mixed with each of the four given quantities of group-specific substances of Witebsky (I-IV) and diluted with saline solution to make a total volume of 1.5 cc. After standing at 37 C. for thirty minutes, each of the four mixtures of two different anti-Rh serums (groups A and O) were tested with three bloods one of which is in group B, Rh negative. The tests were incubated for one hour at 37 C., and readings were made by the centrifugation and resuspension technic

	Anti-Rh Serum, Group A, Witebsky's Substances				Anti-Rh_1 Serum, Group O, Witebsky's Substances			
2% Blood Suspension	0.125 Cc. I	0.25 Cc. II	0.5 Cc. III	1.0 Cc. IV	0.125 Cc. I	0.25 Cc. II	0.5 Cc. III	1.0 Cc. IV
B, Rh⁻	±	—	—	—	+	±	±	—
O, Rh_1	++	++	++	++	++±	++±	++±	++±
O, Rh⁻	—	—	—	—	—	—	—	—

The signs ±, +, ++ and++± indicate varying degrees of agglutination.

27. At the suggestion of Dr. A. W. Frisch, specimens of the blood of this patient were sent by Drs. H. R. Mooi (Union City, Mich.) and G. W. Schelm (Kellogg Foundation Hospital).

28. Levine, P.: Cincinnati J. Med. **23**:596. Landsteiner and Wiener.[15a]

29. Levine[1] employs the A and B group-specific substances originally suggested by E. Witebsky, N. C. Klendshoj and P. Swanson (J. A. M. A. **116**:2654, 1941) in order to make all group 0 bloods safe as universal donor blood by specific neutralization of the normal isoagglutinins. A. S. Wiener and S. Forer (Proc. Soc. Exper. Biol. & Med. **47**:215, 1941) recommended the addition of saliva of group A and B persons (secretor type) for the inhibition of anti-A and anti-B agglutinins in human anti-Rh serums.

agglutinins. As an alternative procedure, one may resort to absorption of the anti-B (or anti-A) isoagglutinins with Rh-negative blood of group B (or A). However, specific absorption requires comparatively large quantities of rare bloods which may not always be available. Additional disadvantages are (1) the necessity of preventing contamination of the absorbed fluid and (2) the requirement of larger quantities of serum for absorption than for inhibition.

Occasional anti-Rh serums were observed to exhibit stronger reactions in higher than in lower dilutions. This effect, known to the immunologist as the "zone phenomenon," is peculiar to only certain serums. Although a particular serum exhibiting this unusual property may be useful if suitably diluted, it should not be distributed for routine testing. Similar observations were made by British workers.[30]

TABLE 5.—*The Zone Effect*

2% Blood Suspension (Group O)	Agglutination with Anti-Rh' Serum (Sil.) Given Group O, Inhibited Dilution of *					
	1:1	1:5	1:20	1:40	1:80	1:160
Rh_1	+	++	++	+	±	—
Rh_1	—	—	+	—	—	—
Rh_1	—	Trace	+	—	—	—
Rh_2	±	+±	+	Trace	—	—
Rh_2	—	Trace	+	+	±	—
Rh_2	+	++	++±	+±	±	—

* These tests were incubated for one hour at 37 C., and readings were made by the centrifugation and resuspension technic. "Sil." in parentheses is an abbreviation of the patient's name.

Finally, anti-Rh serums selected for diagnostic purposes should be free from other atypical agglutinins, most frequently of the cold variety.[31] On several occasions I have observed moderately active or even potent anti-Rh serums which contained also other antibodies active on Rh-negative bloods even at room temperature. In these cases the isoimmunization had been induced by repeated transfusions, and the patient was acutely ill in contrast to the mothers with Rh-negative blood who produce anti-Rh agglutinins.

Actually potent anti-Rh serums which can be recommended as diagnostic reagents are exceedingly rare, so that the production of an equally satisfactory experimental serum would be highly desirable. Even if such a serum becomes available, it will still be necessary to collect and store potent anti-Rh serums of human origin. The present indications are that the experimental anti-Rh serum corresponds in specificity with human anti-Rh serum but certainly not with anti-Rh_1 serum. While a potent agglutinating experimental serum will suffice for the analysis of the greater part of the material to be investigated, evidence of isoimmunization in the exceptional cases may be obtained only with the aid of human anti-Rh serums of diverse specificities.

In the absence of a suitable experimental serum the question of a supply of potent diagnostic anti-Rh serums sufficient to meet civilian and military requirements is most urgent. Undoubtedly the Rh factor is of greater significance in civilian than in military practice. In military practice its importance will depend on the number of wounded persons who will require repeated blood transfusions. As already indicated, there will, as a rule, be a warning, since a particular transfusion will cause a mild or moderately severe reaction. This should serve as a signal for the carrying out of tests with anti-Rh serums. In giving subsequent transfusions to patients whose blood has been found to be Rh negative only Rh-negative blood should be used. Accordingly, it will not be necessary to determine the reactions of the blood of the entire military personnel, but a list of prospective donors of Rh-negative blood should be prepared.

SUMMARY

The indications are that a person with Rh-negative blood who has been immunized either by repeated transfusions or by pregnancy remains immunized for many years if not permanently. It is probable that minute quantities of fetal blood, sufficient to induce isoimmunization, enter the maternal circulation in every normal pregnancy.

Human anti-Rh' or anti-Rh (but not anti-Rh_1) serums are satisfactory for clinical purposes. Human anti-Rh serums must be standardized not only for potency but for specificity. The specificity of human anti-Rh serums depends on the differences in the antigenic composition of the various subtypes of the Rh factor of the blood. The tests, procedures and criteria given will aid in the selection of potent specific diagnostic human anti-Rh serums.

The Rh factor is of importance in military practice because of the frequency with which wounded persons must be given repeated blood transfusions.

30. The Determination of Blood Groups, Medical Research Council War Memorandum no. 9, London, His Majesty's Stationery Office, 1943.

31. Wiener, A. S., and Forer, S.: Proc. Soc. Exper. Biol. & Med. **47**:215, 1941. Vogel and others.[24] Levine and others.[22]

GENESIS OF ENCEPHALOPATHY DUE TO ARSPHENAMINE

(CENTRAL VASOPARALYSIS DUE TO ARSPHENAMINE)

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CINCINNATI

Neurologic complications leading to coma and death following the intravenous administration of arsphenamine have been described under the names of "brain purpura," "hemorrhagic encephalitis" and "perivascular hemorrhage." These different titles express the idea that among the underlying pathologic conditions small diffusely scattered hemorrhages are predominant.

Few observers have described in addition nonhemorrhagic lesions in the brain.[1] But even these nonhemorrhagic alterations have been related to vascular disturbances by some observers.

This presentation was stimulated by a study of 5 cases of encephalopathy due to arsphenamine in all of which hemorrhages and typical vascular alterations were predominant. The vascular lesions were strikingly similar to those recently described in cases of injury of the brain and spinal cord under the heading of "central vasoparalysis."[2] Vascular lesions of the same type have frequently been observed in cases of posttraumatic edema, massive hemorrhage and posttraumatic hemorrhagic infarction.[3]

The frequent occurrence of hemorrhages in cases of encephalopathy due to arsphenamine makes it essential to know their genesis. The purpose of this presentation is to describe from the standpoint of morphology the characteristic vascular change causing hemorrhages and to point out a possible pathophysiologic mechanism.

From the Laboratory of Neuropathology, Cincinnati General Hospital, and the University of Cincinnati College of Medicine.

1. (*a*) Busse, O., and Merian, L.: München. med. Wchnschr. **59**:2330, 1912. (*b*) Schmorl, G.: ibid. **60**: 1685, 1913. (*c*) Russel, D. S.: J. Path. & Bact. **45**:357, 1937. (*d*) Alpers, B. J.: Arch. Neurol. & Psychiat. **20**:497, 1928. (*e*) Roseman, E., and Aring, C. D.: New England J. Med. **224**:550, 1941. (*f*) Straus, F., and Globus, J. H.: J. Nerv. & Ment. Dis. **72**:557, 1930. (*g*) Scheinker, I. M.: J. Neuropath. & Exper. Neurol. **2**:301, 1943. (*h*) Boyd, D. A., and Nie, L.: Arch. Neurol. & Psychiat. **49**:863, 1943.

2. Scheinker, I. M.: Central Vasoparalysis: A Characteristic Vascular Syndrome and Its Significance in the Pathology of the Central Nervous System, to be published.

3. Evans, J. P., and Scheinker, I. M.: Arch. Neurol. & Psychiat. **50**:258, 1943.

MATERIAL

This paper is based on a study of 5 cases of encephalopathy due to arsphenamine. No attempt will be made to describe the findings in each case, but a general description will be given covering the 5 cases. Two illustrative cases will be presented in detail.

CASE 1

A 21 year old unmarried white man was admitted to the hospital because of a sudden onset of delirium following a second intravenous injection of arsphenamine. About two weeks prior to admission, during a physical examination for the Army, it was discovered that the blood gave a positive Wassermann reaction. On this basis antisyphilitic therapy was begun. He received the first injection of arsphenamine (0.5 Gm.) one week prior to admission to the hospital and the second (0.7 Gm.) forty-eight hours before admission. He was admitted to the hospital in a comatose state. On that day he had suddenly become restless, delirious, disoriented and dysarthric.

During the admission examination he tossed about violently in bed, mumbling incoherently and completely unresponsive. The pupils were dilated and reacted to light. The optic fundi were normal. There was increased resistance to passive movements of the extremities. The tendon reflexes in both legs were hyperactive. The abdominal reflexes were normal. There was mild stiffness of the neck, and the Kernig sign was slightly positive.

The cerebrospinal fluid was clear and contained 5 white blood cells per cubic millimeter and 33 mg. of protein per hundred cubic centimeters. The colloidal gold curve was 4433332100, and the Wassermann reaction was positive. The initial pressure was 160 mm. of water.

On the day of admission the patient had generalized convulsions, followed by deep coma and rigidity of the extensor muscles. There was marked resistance to flexion of the arms, wrists, knees and ankles. The fingers were flexed in a position suggesting tetany; the feet and the toes were in plantar flexion. There was occasional twitching of the muscles of the right thigh. The pupils were widely dilated, measuring 7 to 8 mm. in diameter. There was some inward deviation of the right eye. While being observed the respirations slowed markedly, and the patient had a generalized convulsion lasting forty-five seconds. He became cyanotic, the tendon reflexes were hyperactive and the Babinski signs were positive on both sides. He declined rapidly and died on the second day after admission.

Necropsy.—The pathologic changes exclusive of those in the nervous system were: multiple subpleural and small pulmonary hemorrhages; acute bronchitis and enteritis; toxic splenitis and toxic nephrosis.

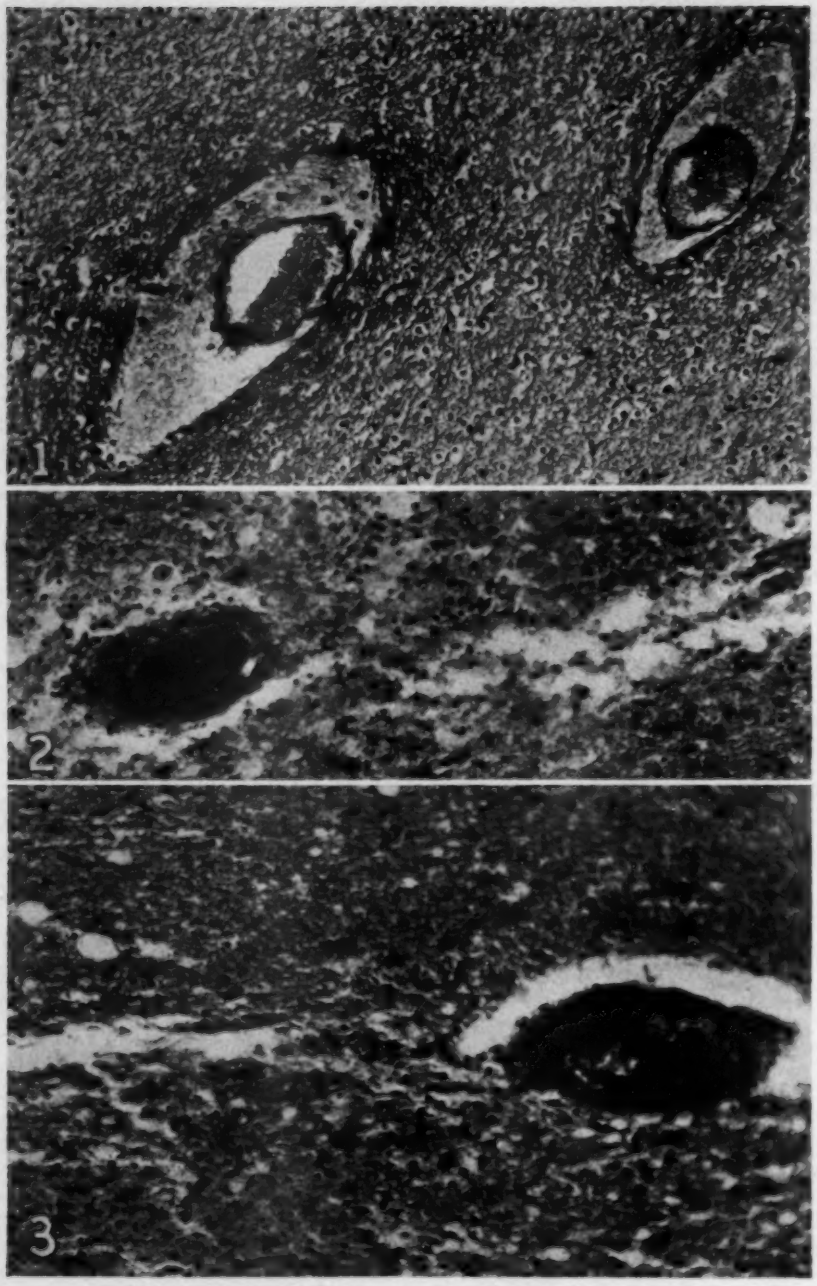
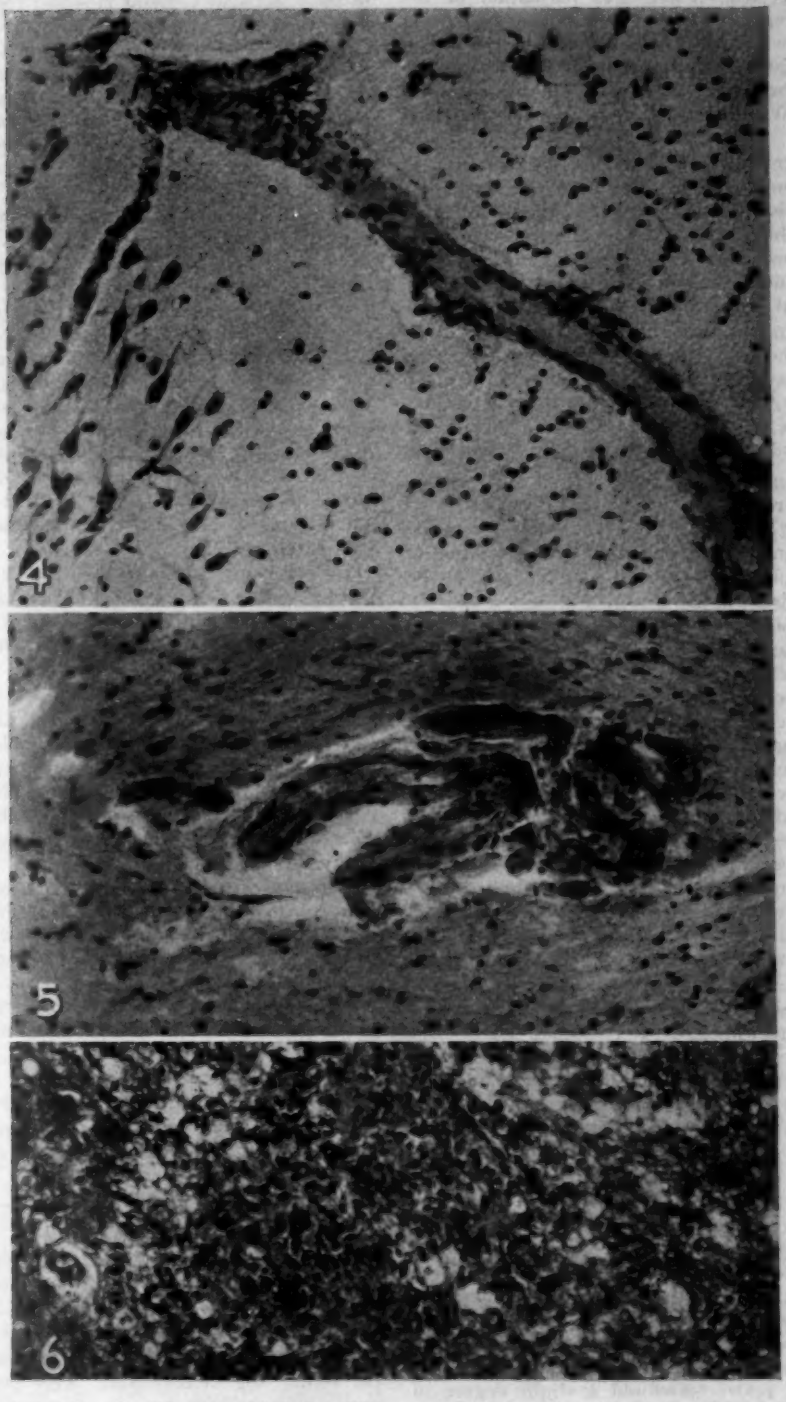


Fig. 1 (case 1).—Distended smaller blood vessels with signs of stasis. Note the degenerative alterations of the vessel walls associated with an increased permeability allowing serous fluid and red blood cells to escape into the maximally distended perivascular spaces. Hematoxylin-eosin stain; × 165.

Fig. 2. (case 1).—Stasis and congestion of a small blood vessel. Note the circumscribed area of beginning rarefaction and necrosis of tissue in the direct proximity of the vessel. Cresyl violet stain; × 165.

Fig. 3 (case 1).—Beginning necrosis and edema of the nerve tissue in the vicinity of a tremendously distended small blood vessel with signs of stasis. Cresyl violet stain; × 165.



Figs. 4 and 5 (case 1).—Proliferation of the capillary endothelial cells. Note the devastated areas of nerve tissue with dropping out of ganglion cells in the vicinity of the vessels.

Fig. 6 (case 1).—Circumscribed area of reactive gliosis characterized by a dense accumulation of glial cells. Hematoxylin-Van Gieson stain; × 165.

The examination of the nervous system was limited to the brain, which weighed 1,550 Gm. and appeared swollen. The convolutions were flattened, and the sulci were shallow. The superficial veins were markedly dilated and congested. There was herniation of the uncus of the temporal lobe on both sides, measuring about 8 mm. in width, with a well marked indentation from the free edge of the tentorium. There was a suggestion of a cerebellar pressure cone. The blood vessels of the circle of Willis were of normal distribution and were soft.

The most striking feature of the sectioned brain was the presence throughout the white matter of small pinpoint areas of discoloration probably due to diffusion of extravasated blood. Similar areas of discoloration were especially numerous in the corpus callosum. The sectioned cerebellum and brain stem showed only a few scattered petechial hemorrhages.

Sections were taken from several hemorrhagic areas of the cerebral hemisphere, the brain stem, the pons and the cerebellum. All sections were stained with the hematoxylin-eosin, cresyl violet, Loyez myelin sheath, Bodian 1 per cent protargol (strong protein silver) and scarlet red methods.

Histologic Examination.—Two principal types of abnormality were revealed: (1) a hemorrhagic lesion associated with a special form of vascular alteration (vasoparalysis) and (2) a nonhemorrhagic alteration of the nerve tissue proper secondary, it is thought, to the circulatory disturbance.

The main histologic changes of the vascular system may be demonstrated by reference to photomicrographs (figs. 1 to 3). What appeared to be pinpoint areas of bloody discoloration to the naked eye were recognized on low power microscopic magnification as small perivascular hemorrhages (fig. 1). The majority of the smaller veins and capillaries appeared tremendously distended and disclosed typical signs of stasis (figs. 1, 2 and 3). Some of the maximally congested veins disclosed definite degenerative alterations of their walls with disorganization of their normal structure. These changes were usually associated with increased permeability of the walls for serous fluid and red blood cells. Many of the smaller blood vessels showed the characteristic picture of diapedesis of erythrocytes and leukocytes.

While these lesions constituted the main alterations of the vessels of the white matter, vascular changes of another type were to be seen in the gray substance. They were chiefly confined to the capillaries and arterioles of the cortical ribbon and the brain stem and consisted of proliferation of the endothelial cells (figs. 4 and 5).

Changes of the nerve tissue proper, secondary to the vascular alterations, were as follows: 1. In the direct proximity of more severely damaged blood vessels there were circumscribed foci of necrosis and rarefaction of tissue (figs. 2 and 3). 2. In some areas there were circumscribed lesions characterized by a dense accumulation of glial cells in the form of reactive gliosis (fig. 6). 3. In preparations stained with special silver stains (Bodian) the nerve fibers in areas adjacent to the smaller blood vessels disclosed a slight degree of swelling and dissolution of some of the axis cylinders. 4. These areas when studied by means of the myelin sheath stain (Loyez) revealed slight perivascular demyelination with slight degeneration of some of the myelin sheaths. 5. In sections taken from the cerebral cortex and the brain stem there were diffusely scattered devastated foci with dropping out of nerve cells. In the centers of some of these areas there were to be seen capillaries with proliferation of the endothelial layer (figs. 4 and 5).

CASE 2

A 39 year old white man was admitted Oct. 22, 1941 to the Cincinnati General Hospital complaining of dyspnea, weakness, palpitation, nosebleeds and increasing pallor of the skin. These complaints had been present for the two weeks preceding admission and had rapidly increased in severity since the onset. He had been receiving antisyphilitic therapy since May 1940. He had received 30 injections of arsphenamine (each of 0.9 Gm.) and 40 injections of a bismuth compound from June 7, 1940 through May 9, 1941.

On admission the patient had pallor of the skin, generalized lymphadenopathy, slight enlargement of the liver and a palpable spleen. The optic fundi showed "flame hemorrhages."

A lumbar puncture Dec. 26, 1940 revealed an initial pressure of 150 mm. of water. The cerebrospinal fluid was clear and colorless and contained 3 lymphocytes per cubic millimeter. The total protein content was 43 mg. per hundred cubic centimeters. The Wassermann reaction of the cerebrospinal fluid and the colloidal gold curve were negative. The bone marrow showed the alterations seen in pancytopenia due to drug sensibility (arsphenamine). The last red blood cell count (Feb. 4. 1942) was 2,270,000, and the white cell count was 1,900.

Despite active therapy (the patient received thirty-three blood transfusions and several marrow transfusions) the course was steadily downhill. In the last few days of life he had bleeding from the gums and a massive subconjunctival hemorrhage of the right eye. He died on Feb. 23, 1942.

Necropsy.—The pathologic observations exclusive of those in the nervous system were: pale, yellowish hypoplastic bone marrow; ecchymotic hemorrhages of the epicardium, the myocardium, the pleura, the hepatic capsule and the gastric mucosa; hepatomegaly and splenomegaly; fatty change in the liver; acute hemorrhagic pyelitis.

The examination of the nervous system was limited to the brain. This weighed 1,520 Gm. and showed on its upper surface a patch of subarachnoid hemorrhage in the right sylvian fissure measuring 2 cm. in dorsal-ventral and 4.5 cm. in anteroposterior direction. Otherwise the brain appeared grossly normal. The vessels of the circle of Willis were soft and of normal distribution.

On coronal sections of the cerebral hemispheres there were to be seen scattered pinpoint hemorrhages. These were particularly marked in the corpus callosum (fig. 7) but also stood out prominently in the centrum ovale and in the cortex of the brain.

Histologic Examination.—Vascular alterations were noted similar to those described in detail in case 1. In addition there were numerous diffusely scattered hemorrhagic lesions. It should be emphasized, however, that while in case 1 the perivascular hemorrhages constituted the main hemorrhagic alterations, in this case small hemorrhages of another type, usually known under the term "ring hemorrhage," were predominant. They were characterized by an unstained central area of necrotic tissue surrounded by a large collection of red blood cells. Occasionally a small capillary could be traced to the central portion of the hemorrhagic ring.

Changes of the nerve tissue proper, probably secondary to the vascular alterations, were somewhat different from those seen in case 1 and deserve a more detailed description.

In the direct proximity of the smaller blood vessels focal lesions of the nerve parenchyma were disclosed. The most striking manifestation of the pathologic process was a small focal lesion—a central necrotic area surrounded by a large collection of glial nuclei. The latter usually had a palisade-like arrangement (figs. 8 and 9). Small circumscribed lesions of this type when seen in cross section were generally circular or ovoid. The glial cells arranged in a radial fashion at the periphery of each of the foci varied considerably in size and shape. Many of the foci contained central capillaries (fig. 9). Relatively few were avascular. It appeared probable that an associated vessel might have been found in each lesion if serial sections had been made. In these capillaries the endothelial layer usually appeared normal. Inflammatory phenomena and hemorrhages were not found in or around these lesions. In the areas of focal alteration of tissue nerve cells, myelin sheaths and nerve fibers had practically disappeared.

Another type of disseminated focal lesion consisted of scattered areas of focal necrosis characterized by complete loss of myelin and coagulation of the immediately contiguous nerve parenchyma (fig. 10). In contradistinction to the previously mentioned areas of necrosis there was no evidence of glial reaction. They were usually sharply circumscribed and acellular. No fat granule cells and no inflammatory reaction could be disclosed. Occasionally some of the lesions suggested a perivascular distribution. The vessel wall usually showed almost complete degeneration.

There were a few scattered areas of perivascular rarefaction and edema of tissue (fig. 11). In their structure they resembled the lesions described as miliary anemic infarction. No formation of compound granular cells was observed.

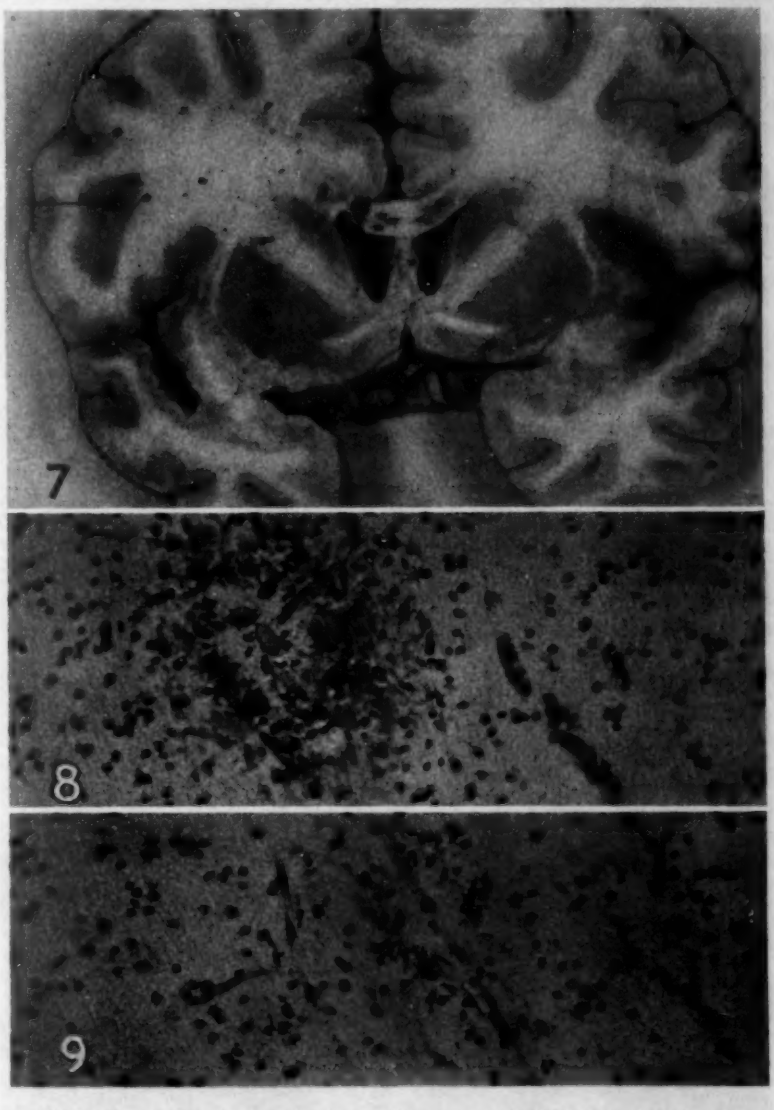


Fig. 7 (case 2).—Diffusely scattered pinpoint hemorrhages, particularly marked in the corpus callosum.

Fig. 8 (case 2).—Small focal lesion of necrosis surrounded by a large collection of glial nuclei arranged in a palisade-like fashion. Cresyl violet stain; × 165.

Fig. 9 (case 2).—Small area of necrosis with a central capillary. Cresyl violet stain; × 165.

SUMMARY OF THE PATHOLOGIC CHANGES CHARACTERIZING ENCEPHALOPATHY DUE TO ARSPHENAMINE

It appears that the most constant and uniform pathologic alteration in the brain in cases of encephalopathy due to arsphenamine is a vascular lesion described recently under the heading of central vasoparalysis.[2] The morphologic criteria for this vascular syndrome were listed as follows: (1) maximal distention and engorgement of the smaller veins and capillaries with blood and associated signs of stasis; (2) degenerative changes or complete necrosis of the vessel wall with an increase of permeability for serous fluid and red blood cells; (3) distention of the perivascular spaces, which usually harbor extravasated fluid and red blood cells.

In the previous description of the syndrome "central vasoparalysis" it was pointed out that these vascular changes may occur as a result of the presence of an acute physical or chemical irritant. Trauma seems to be of special significance. In a systematic study of lesions of the central nervous system following trauma,[3] similar vascular lesions were shown to be predominant. Vascular alterations of the same type were observed repeatedly in cases of edema of the brain.[4]

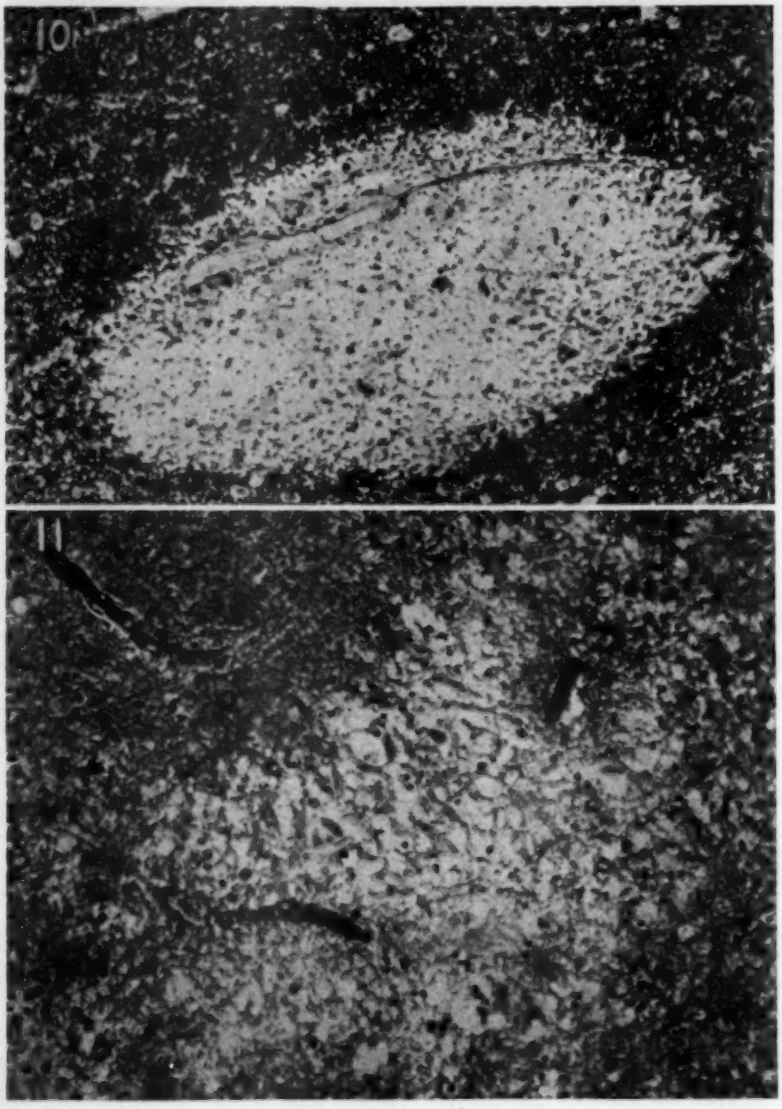


Fig. 10 (case 2).—Sharply circumscribed foci of loss of myelin with coagulation necrosis of the nerve parenchyma. Note the complete absence of glial reaction. Hematoxylin-eosin stain; × 135.

Fig. 11 (case 2).—Small area of perivascular rarefaction and edema of tissue (anemic infarction). Hematoxylin-eosin stain; × 165.

4. Scheinker, I. M.: Deutsche Ztschr. f. Nervenh. **147**:137, 1938; Arch. Neurol. & Psychiat. **45**:117, 1941.

Among the underlying pathologic conditions in cases of encephalopathy due to arsphenamine a diffuse scattering of small hemorrhagic lesions has been predominant.

Hemorrhages of different types are observed resulting from the administration of arsphenamine. Those most frequently observed are minute punctate hemorrhages distributed chiefly in the white matter, especially in the corpus callosum and the centrum semiovale. These small hemorrhages usually occur in the forms of (*a*) petechial or perivascular hemorrhages filling the Virchow-Robin spaces and (*b*) so-called ring hemorrhages with hemorrhagic infiltration of the tissue adjacent to the vessel. Their nature has been discussed by Globus and Ginsburg,[5] who explained their morphologic peculiarities on the basis of "selective damage to the capillaries."

While the vascular alterations associated with small hemorrhagic lesions constituted the main cerebral changes in cases of encephalopathy due to arsphenamine, a series of nonhemorrhagic lesions were disclosed in the nerve parenchyma adjacent to the smaller vessels. These nonhemorrhagic lesions can be summarized briefly as follows: (1) areas of necrosis of tissue in the direct proximity of severely damaged blood vessels (fig. 2); (2) circumscribed patches of dense cellular gliosis (fig. 6); (3) small foci of rarefaction and edema of tissue (fig. 11); (4) devastated areas of the cortical ribbon in the proximity of capillaries with hypertrophy and hyperplasia of their endothelial cells (fig. 4); (5) patches of demyelination associated with circumscribed coagulation of the nerve parenchyma (fig. 1 *D*)—a type of nonhemorrhagic alteration of tissue which was described in detail in a previous communication[1g]; (6) a small pericapillary focus of necrosis surrounded by a large ring of glial nuclei in palisade arrangement (figs. 8 and 9). This last type of perivascular necrosis has been described by Alpers.[1d]

THE PATHOPHYSIOLOGIC MECHANISM OF THE HEMORRHAGIC AND NONHEMORRHAGIC ALTERATIONS TYPICAL OF ENCEPHALOPATHY DUE TO ARSPHENAMINE

Knowledge of the precipitating mechanism of the hemorrhagic lesion is limited. Globus and Ginsburg[5] have advanced the view that a direct and selective effect of the arsphenamine on the vascular endothelium is responsible for the disruption of the capillaries. According to Globus and Ginsburg, the hemorrhages are due to "rupture of the smaller capillaries." The view has been frequently expressed that the cerebral complications following the administration of arsphenamine are due to the Herxheimer reaction. This explanation does not seem to be correct inasmuch as the same cerebral alterations have been described in nonsyphilitic subjects (Globus and Ginsburg[5]).

Boyd and Nie,[1h] reporting histologic observations in 4 cases of encephalopathy due to arsphenamine, attributed the hemorrhagic and the nonhemorrhagic focal lesions to "a common vascular basis." Congestion and stasis of the smaller vessels associated with edema, focal necrosis and capillary hemorrhages were the most characteristic changes observed.

Tannenberg and Fischer[6] suggested that the dysfunction of the cerebral blood vessels resulting in hemorrhages is due to the action of the arsphenamine on the nerve terminals in the cerebral vessels.

Different theories have been offered in attempting to explain the origin of the nonhemorrhagic alterations of nerve tissue. Alpers[1d] suggested the following possibilities: (1) As a result of injury to the vessels and occlusion of the small capillaries there may develop an area of coagulation necrosis immediately surrounding the vessels; (2) as a result of injury to the endothelium of the capillary vessels by a toxic or an infectious process, neuroglia cells are collected about the vessels. The central necrosis of the foci is explained by the "occlusion of the vessel by the swollen endothelium or possibly by a thrombus." Russel,[1e] who described focal lesions similar to those observed in cases of encephalomyelitis after smallpox and measles, suggested the possibility that the lesions are a phenomenon of sensitization. Oeller[7] explained the nonhemorrhagic lesions by venous thrombosis with damming back of blood. Schmidt[8] advanced the theory that the alterations of nerve tissue were due to perivascular hemorrhages which resulted from diapedesis of red blood cells following injury to the blood vessel or following circulatory stasis.

In final analysis, all the histologic alterations described within the nerve parenchyma are to be considered as secondary to local circulatory disturbances. This view has been widely accepted. It seems, therefore, that the histologic changes described in smaller blood vessels provide the important clue to the pathophysiologic mechanism.

5. Globus, J. H., and Ginsburg, S. W.: Arch. Neurol. & Psychiat. **30**:1226, 1938.

6. Tannenberg, J., and Fischer, B.: Frankfurt. Ztschr. f. Path. **33**:91, 1925.

7. Oeller, H.: Deutsche Ztschr. f. Nervenh. **47**:504, 1913.

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The following theory of the mechanism is suggested. The main vascular alterations observed in all cases of encephalopathy caused by arsphenamine correspond to those lesions described under the heading of central vasoparalysis.[2] These vascular lesions occur as a result of the presence of an acute physical or chemical irritant, and trauma seems to be the most frequent etiologic factor. The researches of Cohnheim,[9] Ricker[10] and others have demonstrated that profound circulatory disturbances may result from chemical or physical stimuli applied to the vessel wall and may be propagated along the affected vessel. After a contraction of the blood vessel wall of short duration there soon occurs a definite paralytic dilatation of the exposed vessel. This is usually associated with stasis; an apparent slowing of the blood stream occurs. If the circulation is impaired because of stasis, a local accumulation of carbon dioxide would result and hence further vascular dilatation. The retarded circulation would no doubt interfere with the adequate supply of oxygenated blood to smaller veins and capillaries. If these alterations are sufficiently severe, degeneration of the vessel wall may occur. The vessel wall would become more permeable for serous fluid and red blood cells. The resulting extravasation of large masses of red cells into the tremendously distended perivascular spaces would form the punctate hemorrhages. The engorgement of the perivascular spaces with blood would serve in turn further to retard the circulation and contribute to the hypoxia of the nerve tissue. There is no doubt that these changes would interfere with proper exchange of oxygen and carbon dioxide, so that the metabolism of the tissue would be more and more disturbed and finally incompatible with the maintenance of the normal function. This would lead to pericapillary necrosis of tissue, the different forms and stages of which have been described.

In support of this conception the following points may be marshaled: 1. It is generally known that arsphenamine may act as a vasodilator. The greater concentration of the drug in smaller veins and capillaries caused by the stasis may increase the pharmacodynamic property of the drug in its action on the capillary walls. 2. Siengalewicz[11] showed in his experimental studies that arsphenamine causes increased permeability of the capillaries of the brain in rabbits. These animals were given intravenous injections of trypan blue and then were given large doses of arsphenamine. The trypan blue escaped into the nerve parenchyma and diffusely stained the white matter about the lateral ventricles. 3. Ecker and Kernohan[12] expressed the view that "vasomotor paralysis leads to engorgement of the smaller vessels and to edema of the central nervous system. Subsequently fatty degeneration of the endothelium of these vessels leads to the formation of thrombi and perivascular changes, namely necrosis, gliosis and hemorrhages."

No real thrombus formation was observed in our cases. The vessels showed only signs of stasis and engorgement.

TERMINOLOGY

The frequently used term "hemorrhagic encephalitis" should be abandoned. The lesions of the brain are not inflammatory as the term "encephalitis" might imply. The term "hemorrhagic" covers only a part of the alteration of tissue. The term suggested by Alpers, "medullary perivascular necrosis," would not seem to cover the vascular lesions and hemorrhage, which form one of the most characteristic features of the process. Globus suggested the term "pericapillary encephalorrhagia due to arsphenamine" which is more explanatory than "hemorrhagic encephalitis" but does not consider the nonhemorrhagic alterations, which no doubt are essential in the disorder.

Therefore, it seems that "encephalopathy due to arsphenamine" is adequate. This term indicates that the pathologic process is not an inflammatory one and at the same time covers the vascular, hemorrhagic and nonhemorrhagic alterations of tissue.

SUMMARY

The essential alterations in the central nervous system in 5 cases of encephalopathy due to arsphenamine consisted mainly of vascular changes typical of central vasoparalysis, associated with focal hemorrhagic and nonhemorrhagic lesions in the nerve parenchyma.

The vascular changes observed in the smaller veins and capillaries in these cases and described in detail suggest a possible pathophysiologic mechanism. The widely disseminated focal lesions (hemorrhagic and nonhemorrhagic) appear to be secondary to the vascular alterations.

In view of the vascular nature of the main alterations, the term "encephalopathy due to arsphenamine" appears more appropriate than "hemorrhagic encephalitis" or "brain purpura."

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10. Ricker, G.: Virchows Arch. f. path. Anat. **226**: 180, 1919.

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EOSINOPHILIC GRANULOMA OF BONE

A CONDITION AFFECTING ONE, SEVERAL OR MANY BONES, BUT APPARENTLY LIMITED TO THE SKELETON, AND REPRESENTING THE MILDEST CLINICAL EXPRESSION OF THE PECULIAR INFLAMMATORY HISTIOCYTOSIS ALSO UNDERLYING LETTERER-SIWE DISEASE AND SCHÜLLER-CHRISTIAN DISEASE

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In 1940 we[1] described cases presenting a solitary bone lesion which we regarded as being inflammatory in nature and whose histologic peculiarities seemed to be economically expressed in the name "eosinophilic granuloma of bone." Independently of us, Otani and Ehrlich[2] described, under the name of "solitary granuloma of bone," cases showing the same type of lesion, and they leaned toward the idea that trauma might be the initiating cause of it.

In the cases which we reported we found the lesion to be characterized histologically by conspicuous sheetlike collections of histiocytes (including some revealing phagocytic activity), interspersed among which there were more or less prominent accumulations of eosinophilic cells and especially eosinophilic leukocytes. In addition, however, we usually also saw fields of hemorrhage and necrosis and, particularly in relation to these fields, larger or smaller numbers of multinuclear giant cells, some of which likewise showed phagocytic activity. The modifications which may be superimposed on this picture —for instance, by the scarring effects of a fracture at the site of the lesion—will be brought out later. In any event, we were led to apply the name "eosinophilic granuloma of bone" to the lesion because of the imprint which the eosinophils give it, though we recognized that its basic cells are the histiocytes.

Not long after publication of our paper, we became aware that one may also encounter cases with multiple bone lesions of the same kind as the solitary lesion just mentioned. The case which first brought about this realization showed lesions in a number of bones roentgenographically, though only one of them was giving rise to clinical complaints when the patient was first admitted to our hospital. It thus also became clear that even in cases in which supposedly there is only a solitary lesion, lesions may be present in other bones too, though silent, and should be searched for roentgenographically.

Farber[3] confirmed the occurrence of cases both with solitary and with multiple skeletal lesions such as had been described by Otani and Ehrlich and by us. In the subsequent fuller report by Green and Farber[4] the clinical peculiarity of these cases was stressed and, on the pathologic side, the peculiar granulomatous character of the lesion's tissue was emphasized. Indeed, Green and Farber, too, pointed out the necessity for singling out these cases on the basis of their special character.

In such cases the skeletal lesions (whether single or multiple) certainly occupy the foreground of the disease picture. Farber, though agreeing that clinically the cases should be singled out as belonging in a group by themselves, held that anatomically the underlying lesion[5] in them is related to the lesion of Schüller-Christian disease and that of Letterer-Siwe disease. The idea that the latter two diseases are anatomically interrelated was not new, having previously been propounded by Flori and Parenti,[6] Glanzmann[7] and Wallgren,[8] for instance. However, the idea of interrelating the lesion of what we call eosinophilic granuloma of bone to that of Letterer-Siwe disease and that of Schüller-Christian disease was new and con-

From the Laboratory Division of the Hospital for Joint Diseases.

1. Lichtenstein, L., and Jaffe, H. L.: Am. J. Path. **16**:595, 1940.

2. Otani, S., and Ehrlich, J. C.: Am. J. Path. **16**: 479, 1940.

3. Farber, S.: Am. J. Path. **17**:625, 1941.

4. Green, W. T., and Farber, S.: J. Bone & Joint Surg. **30**:499, 1942.

5. It was called "destructive granuloma of bone" by Green and Farber, "solitary granuloma of bone" by Otani and Ehrlich, and "eosinophilic granuloma of bone" by us and also by C. H. Hatcher (Arch. Path. **30**:828, 1940) and T. B. Mallory (Eosinophilic Granuloma of Bone, Cabot Case 26362, New England J. Med. **223**:149, 1940).

6. Flori, A. G., and Parenti, G. C.: Riv. di clin. pediat. **35**:193, 1937.

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stituted what we now agree was a forward step toward the understanding of the larger subject.

Altogether, it would now seem that eosinophilic granuloma of bone, Letterer-Siwe disease and Schüller-Christian disease constitute different clinical expressions of the same basic disorder, which seems to predilect the hemopoietic system. Its lesions apparently represent a peculiar inflammatory reaction to some as yet unknown infectious agent and are characterized cytologically at their start by the presence in them of large numbers of histiocytes. A corollary of this thesis is the idea that one must remove Schüller-Christian disease from the category of primary disorders of lipid metabolism. Indeed, it is not Schüller-Christian disease but only xanthoma tuberosum multiplex that constitutes the cholesterol lipid disorder analogue of Gaucher's disease and Niemann-Pick disease.

A useful schematization of the clinical gradations of expression of the basic disorder underlying Letterer-Siwe disease, Schüller-Christian disease and eosinophilic granuloma of bone was given by Mallory.[9] We are in general accord with it, while recognizing that there are some cases, such as the one reported by Currens and Popp,[10] which present problems of classification within it. Its essential features are retained in the following restatement: 1. The disorder may manifest itself in infancy or very early childhood in an often rapidly fatal form in which the histiocytic lesions are widely distributed both through the soft tissues (especially the lymphoid tissues) and the skeleton (so-called Letterer-Siwe disease, reticulosis or nonlipoid histiocytosis). 2. The disorder may appear in children or adults (even middle-aged ones) in a chronic form in which the histiocytic lesions, again usually not limited to the skeleton, tend to undergo collagenization and lipidization, and in which the prognosis is often still grave because of the likelihood of damage to the lungs and heart and to the brain and pituitary gland (Schüller-Christian disease or lipogranulomatosis). 3. The disorder may appear in children or young adults in a comparatively benign and much more localized form in which the lesions seem to occur only in the skeleton (and often only in a single bone), heal readily after simple curettage even without supplementary radiation therapy, and may indeed heal by resolution even without any therapeutic intervention (eosinophilic granuloma of bone).

In this schematization, the term "eosinophilic granuloma of bone" comes to designate a clinicoanatomic category instead of referring merely to the histologic picture of an individual lesion in this category, as it did in our original article. One may ask why we are retaining this name in preference to using "solitary granuloma of bone" as proposed by Otani and Ehrlich or "destructive granuloma of bone" as proposed by Green and Farber. In the first place, we now know that in cases of eosinophilic granuloma of bone the disease process may be present not only in one bone but in several or even many bones, so that the designation "solitary granuloma" is already inappropriate on this score alone. Furthermore, the term "solitary granuloma" is nondescript, leaving out, as it does, the idea of the eosinophils which hallmark the lesions histologically.

Some of these objections, and still others, can also be raised against the phraseology used by Green and Farber for cataloging these cases, to wit: "destructive granuloma of bone, single or multiple as the case may be, adding parenthetically Hand-Schüller-Christian syndrome." Specifically, "destructive granuloma of bone, single or multiple" is nondescript, for gummatous syphilitic lesions, for instance, involving one or more bones, are destructive granulomas. Furthermore, adding, even parenthetically, the term "Hand-Schüller-Christian syndrome" is straining a point, even if it was not the intention to focus on the triad of diabetes insipidus, calvarial defects and exophthalmos which is generally accepted as the syndrome in question. Certainly a case presenting an eosinophilic granulomatous lesion in one bone only (and not a calvarial bone at that), in which there are no manifestations of general disease and in which simple curettage leads to complete healing of the condition has *no clinical* affiliation with cases of Schüller-Christian disease.

In regard to the eosinophils in the characterization of the histologic picture it is to be noted that their importance was recognized even in the old articles which misinterpreted cases of the kind in question as "myeloma with prevalence of eosinophile cells"[11] or "osteomyelitis with eosinophile reaction."[12] Furthermore, Green and Farber, referring to the eosinophils,

9. Mallory, T. B.: New England J. Med. **227**:955, 1942.

10. Currens, J. H., and Popp, W. C.: Am. J. M. Sc. **205**:780, 1943.

11. Finzi, O.: Minerva med. **91**:239, 1929.

12. Schairer, E.: Zentralbl. f. allg. Path. u. path. Anat. **71**:113, 1938.

stated that in what they interpreted as the early phase of the lesion under consideration, eosinophils "may be gathered in large clumps or masses in such a way that the field is discolored a bright red by their presence, or they may be scattered diffusely through the entire section." In those 5 of their cases which are detailed under "Case Reports" one finds consistently that the presence of smaller or larger collections of eosinophils is noted in connection with the histologic nature of the biopsy specimens. Green and Farber did state that "in a somewhat older stage of the disease process, eosinophiles are no longer present." This may be true, though our own experience counts against the complete disappearance of the eosinophils before complete healing of the lesion. In any case, however, it seems to us that since they are so consistently numerous, at least in what is regarded as the early stage of the lesion, mention of them in the name helps to fix the lesion in one's mind.

The purposes of the present communication are: to develop the concept of eosinophilic granuloma of bone as a clinicoanatomic category, to redescribe the lesion of eosinophilic granuloma of bone histologically on the basis of wider experience with it and to show how the clinicoanatomic category of eosinophilic granuloma of bone touches on the categories of Schüller-Christian disease and Letterer-Siwe disease.

CLINICAL AND ROENTGENOGRAPHIC FEATURES

Eosinophilic granuloma of bone is seen mainly in children and adolescents, though sometimes also in young adults. As already noted, our original paper concerned itself with cases in which only one bone seemed to be affected, and we have now examined material from 10 cases, in all, of this type. That one may encounter cases in which several or even many bones are involved is now also clear, and our experience to date also includes 2 cases of this type. Of the 10 cases reported on by Green and Farber, 4 showed single and 6 showed multiple lesions, and none of their patients was more than 11 years old, though it should be noted that these cases were reported from the Children's Hospital in Boston.

With the possible exception of the bones of the hands and feet, almost any bone apparently may be the site of a lesion in a case of eosinophilic granuloma whether the involvement of bone is single or multiple. In the cases in which more than a few bones are involved, those affected are likely to include one or more bones of the cranial vault, some of the ribs, one or more of the vertebrae and several of the long bones—especially the humerus and femur. However, in neither of the 2 cases of multiple involvement of bones which we studied did this involvement include the skull. This is interesting in view of the fact that in one of these there were lesions in eight bones. Specifically, when first admitted to the hospital, the patient in the latter case, a girl of 8, showed a lesion in each of the following bones: the right femur, the left femur, and ninth and eleventh right ribs (fig. 1), the second and ninth left ribs and on subsequent admissions the eighth left rib and the seventh cervical vertebra.

Dr. Samuel Kleinberg, of our hospital, under whose care the patient was, manifested a constructive interest in eosinophilic granuloma of bone and was helpful in regard to this case.

The clinical difficulties are usually limited to such local complaints as may arise from the skeletal lesions, systemic manifestations being entirely absent as a rule even in the cases with multiple involvement of bones. However, as already stated, in a case with multiple involvement of bones it may be only one lesion that creates local clinical difficulties, all the others being silent at first or even consistently. Thus, in the case mentioned in the foregoing paragraph in which there were lesions in eight different bones, only one—the one in the neck of the right femur—was causing clinical difficulties on the patient's original admission. Subsequently, involvement of the seventh cervical vertebra led to collapse of the latter, thus becoming an additional source of clinical complaints. The fact that in a given case there can be so many silent lesions definitely suggests that occasional cases, even those with multiple involvement of bones and, a fortiori, cases in which only one bone is affected, may entirely escape detection.

When there are difficulties referable to an affected bone, local tenderness and pain are the most common. Other clinical findings will depend on the part involved. For instance, if the part affected is rather superficial—a rib or the calvarium for example—a local swelling without heat may be palpable. A lesion in the neck of a femur will usually be found associated with a limp, and a lesion in a vertebral body with some local deformity of the spinal column; lesions in other sites may create corresponding local problems.

The patient's complaints may be of only a few days' or of several weeks' or months' standing at the time of admission. In an occasional in-

stance inquiry may reveal that the onset of the complaints was associated with slight fever for a few days. In one of our cases the disease seemed to have been ushered in by an attack of moderate but generalized abdominal pain. (Possibly the gastrointestinal tract was the portal of entry for the infectious agent.)

In an occasional instance, leukocytosis of moderate severity but not associated with any change in the differential count is found. In some instances, also, whether or not there is leukocytosis, the differential count shows a slight increase of the eosinophilic leukocytes in the blood smears. In one of our cases presenting a solitary lesion it was not until one and a half years after the lesion had been curetted and had healed that the eosinophil count (4 per cent on admission) dropped to normal. In the cases in which chemical estimations were made, including those of blood cholesterol, cholesterol esters and total lipid, they yielded normal findings. In the few instances in which tissue from lesions was cultured for bacteria and viruses the results were negative. In a further search for evidences of virus infection, material from two lesions was inoculated into mice, rabbits and guinea pigs by various routes (including the intracerebral and intratesticular), again with negative results.

In general, the individual lesion of bone presents itself roentgenographically as a small or a large radiolucent zone (fig. 1 *A*). In the calvarium the lesions tend to appear more or less circular and rather sharply delimited, the zone of rarefaction even looking punched out. Extensive resorption of the tables, particularly of the outer table, will generally be found associated with swelling of the overlying soft parts. In a scapula or an innominate bone, too, as seen in the roentgenogram the lesion tends to appear as an oval or rather circular, almost punched-out area of rarefaction. In a rib (fig. 4 *A*) the affected area, in addition to being rarefied, may be expanded and also fractured in one place or another, and if fracture is present there may even be evidence of periosteal deposition of new bone and thickening of the overlying soft tissue. As to long bones, too, it will be found that the lesion begins to evolve in the interior of the bone and that as it enlarges to implicate the neighboring cortex the latter becomes eroded from the inside. Furthermore, the cortex may become expanded and even perforated, and where perforation has taken place, deposition of periosteal new bone may be noted (fig. 1 *B*).

Altogether, there is nothing distinctive about the roentgenographic appearance of the individual lesion. For that reason, it is easy to understand why in cases in which the disorder is limited to a single bone the focus of bone destruction as seen in the roentgenogram may, in the light of the clinical picture as a whole (rapid onset, meagerness of general manifestations, etc.), suggest even a primary cancerous growth. In the cases in which several or many bones are involved, the roentgenographic appearances may again suggest a cancerous growth, specifically multiple myeloma, Ewing's tumor or tumor metastatic to the skeleton. Furthermore, and more logically, the roentgenographic picture in these cases (particularly in the presence of lesions in the skull) may suggest Schüller-Christian disease.

PATHOLOGY

It seems to us that there are as yet certain gaps in the present understanding of the natural anatomic life cycle of the lesion of eosinophilic granuloma of bone. There is general agreement as to the anatomy of the lesion in what may be regarded as its early and unmodified form. That is, there is agreement on the microscopic aspects of the lesion as presented within a few weeks or at most three or four months after its appearance and especially when it has not been modified by reparative scarring in response to perforation or fracture of the bone at its site. However, the natural lesion in what may be regarded as a later form (that is, the older, untreated lesion) needs additional exploration from the standpoint of pathology. Indeed, it seems to us that such statements as one finds about it lean more heavily on inference than on direct observation.

Pathology of the Lesion in Its Early Form.—When an affected region of bone (not fractured) in which the lesion is in an early phase is entered, it is likely to be found more or less hemorrhagic and cystic and to show a relatively small amount of soft brownish granulation tissue, which may be streaked with yellow. Indeed much of this granulation tissue may be found necrotic. Characteristically the nonnecrotic tissue presents on microscopic examination, as noted, conspicuous sheetlike collections of large phagocytic cells of the nature of histiocytes, interspersed among which are more or less conspicuous numbers of eosinophilic cells and especially of eosinophilic leukocytes. Furthermore, one finds actively phagocytic multinuclear giant cells, especially in the vicinity of

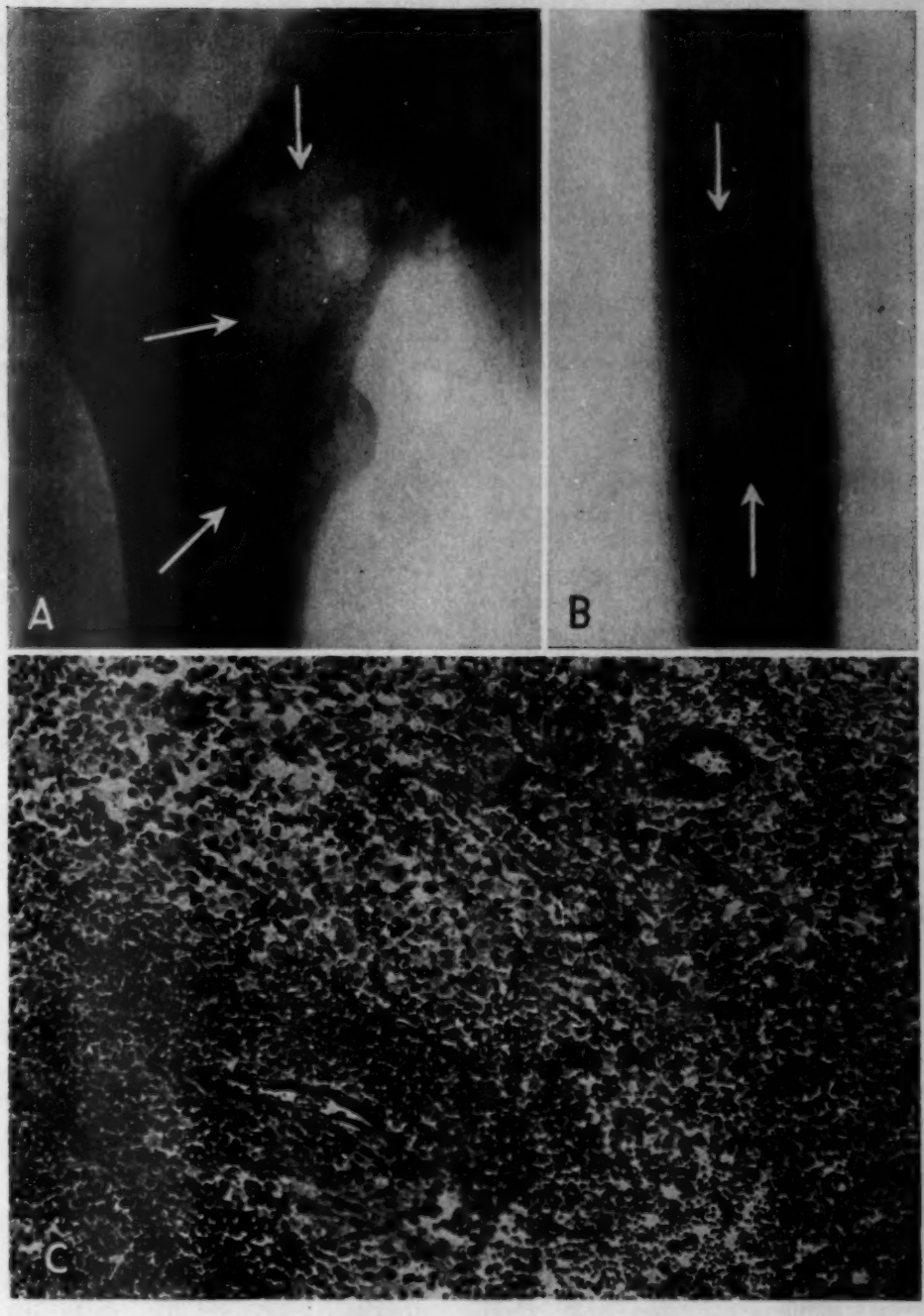

Fig. 1.—*A*, roentgenogram showing a lesion of eosinophilic granuloma of bone in the neck and adjacent area of the shaft of the right femur of an 8 year old girl. The lesion, which is represented by the rarefaction shadow, is threatening the integrity of the cortex of the neck on the medial side. The patient presented lesions in seven other bones also, but it was this femoral lesion that first directed attention to the disease. A roentgenogram taken one month before this one, on account of pain in the hip, showed a lesion at the site in question, but it was then of much smaller extent. It is rather characteristic of lesions of eosinophilic granuloma of bone that they increase fairly rapidly in size.

B, roentgenogram showing a lesion in the upper portion of the shaft of the left femur in the case illustrated in *A*, taken on the same day. This lesion was clinically silent. As revealed by comparison with a roentgenogram of it taken a month earlier, it had likewise increased rapidly in size. Note the periosteal deposition of new bone in response to involvement of the cortex.

C, photomicrograph (× 200) of tissue curetted from the lesion of the femur shown in *A*. In the field illustrated, eosinophils, represented by the concentrated dark cells, are numerous and indeed dominate the picture. The pale cells are histiocytes. The small dark dots to be seen especially at the bottom of the picture, toward the right, represent red blood cells. See also figure 2 *A*.

fields of hemorrhage and necrosis (figs. 1 *C*, 2 and 3 *A*).

As already noted, it is the histiocytes that constitute the basic component of the lesion. Most of them contain a single nucleus, but a small number have two nuclei or even more. The nuclei are large and usually roundish, although some are oval, kidney shaped, creased or otherwise modified in contour (fig. 2 *B*). In the cytoplasm of the uninuclear or multinuclear histiocytes and also in the giant multinuclear cells one often observes phagocytosed erythrocytes and granulocytes (usually eosinophils) in various stages of disintegration, as well as brownish hemosiderin granules (fig. 3 *A*). The leukocytes and hemosiderin particles are readily discernible in preparations stained with hematoxylin and eosin. The red blood cells, though refractile, are not always easy to detect in such preparations but can be seen reasonably well in sections stained with eosin and methylene blue. Particularly in the vicinity of fields of necrosis, some of the unicellular and many of the multicellular or giant histiocytes may be found to contain sudanophilic droplets. These are not doubly refractile, as a rule, and apparently represent phagocytosed particles of the neutral fat naturally present in the invaded interior of the affected bone.

The histiocytes are apparently derived from the multipotent reticulum cells in the adventitia of the blood vessels in the marrow. Indeed, in many of the lesions one finds evidence of plumping and multiplication of the adventitial cells of these vessels. In addition, one can note their apparent transformation into more roundish cells of the general size, shape and character of histiocytes, and their close resemblance subsequently to the neighboring cells of the lesion, which are indubitably histiocytes exhibiting phagocytosis.

As also already remarked, the eosinophils, though not the basic constituent of the lesion, yet give it a striking imprint, especially when present in large numbers (fig. 1 *C*). For the most part, these cells present irregular or bilobular nuclei, though in some lesions or in some places in a given lesion the nuclei of the eosinophilic cells were roundish and not indented (fig. 2 *A*). Incidentally, the small size of these uninuclear eosinophils militates against the idea that they might be eosinophilic myelocytes. (In connection with the finding of the eosinophilic leukocytes in the lesions, it should be pointed out that it may be difficult to demonstrate these cells in tissue which has been left too long in decalcifying fluid.) In some lesions or parts of lesions one may also find some lymphocytes, plasma cells and even neutrophilic polymorphonuclear leukocytes in small numbers.

If a fracture through the site of the lesion has been followed by a reparative reaction to it, the interior of the bone at that site may be found filled in more or less completely by a cellular tissue which is reddish-gray-white on the whole and even tinged with yellow. On histologic examination, however, it will be found that, though the lesion as a whole has undergone connective tissue scarring in many places, it has nevertheless not lost its characteristic features (fig. 3 *B*). That is, one can still see large fields or even delineated foci of tissue dominated by histiocytes which are intermingled with eosinophils and especially with eosinophilic leukocytes. Though the lesion as a whole may be traversed in an irregular way by strands and patches of connective tissue, this tissue can be traced to the fracture because of its association with parosteal scarring, periosteal new bone formation and callus formation. Hence, the presence of scarring under these circumstances should not be taken as indicating that scarring represents a phase in the healing of the eosinophilic granulomatous lesion as such.

Question of Transformation of the Lesion in Its Older Form into Lipogranuloma.—Green and Farber [4] maintained that when the lesion first passes from the early to the older stage, eosinophils are no longer to be found in it, large vacuolated mononuclear cells are seen predominating and the ingrowth of connective tissue is evident. They went on to say that further along in the evolution of the lesion the large mononuclear cells become lipophages and take on the typical appearance of "foam cells." They maintained that still later connective tissue crowds out the mononuclear cells and lipophages and in turn is transformed into bone. We are not entirely clear as to the meaning of the last statement but presume it to mean that there is complete anatomic restitution to the normal state of the local area of bone. Altogether, we would interpret the statement of Green and Farber regarding the progression of the pathologic changes as indicating that the lesion passes from the early or acute stage of a histiocytic granuloma dominated by eosinophils to a stage in which the picture becomes interpretable as that of lipogranuloma (reminiscent of the lesion of Schüller-Christian disease) and that after this the lipogranuloma disappears and the part undergoes anatomic restitution to a normal state.

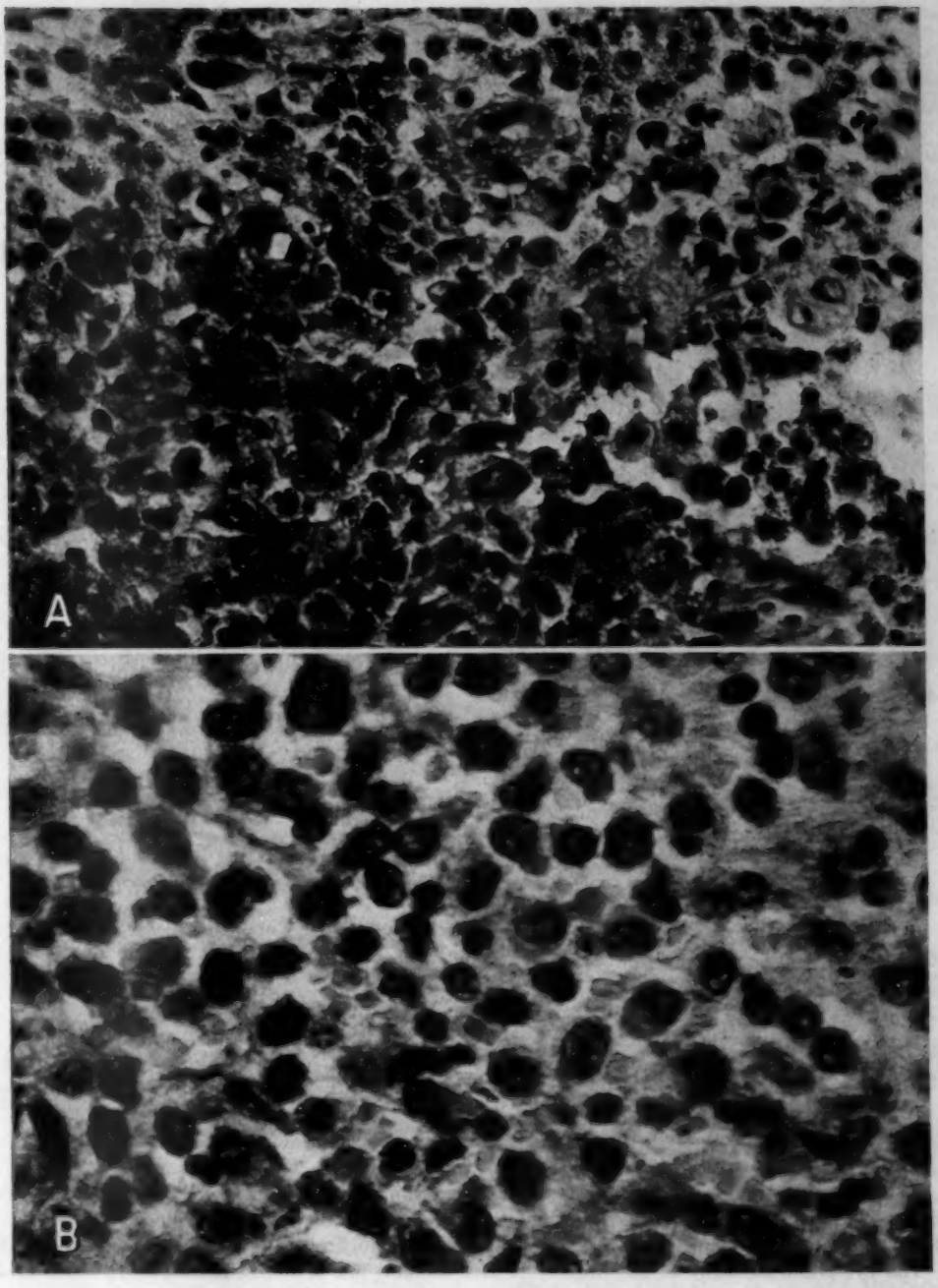

Fig. 2.—*A*, photomicrograph (× 675) of a field near the one from which figure 1 *C* was made. Eosinophils are again numerous. Note the granules in their cytoplasm and also their round, bilobed or even trilobed nuclei. Relatively few histiocytes are seen, and many of these appear to have degenerated.

B, photomicrograph (× 900) showing, in contrast to figure 1 *C* and 2 *A*, a field in which histiocytes dominate the cytologic picture. The presence of sheets of cells with this appearance in field after field may by itself create the erroneous impression that one is dealing with a tumor. However, in other fields from this lesion eosinophils were numerous and indeed again dominated the cytologic picture. The curettings in this case came from a femur of a child of 4 in whom the disease was present in that bone only.

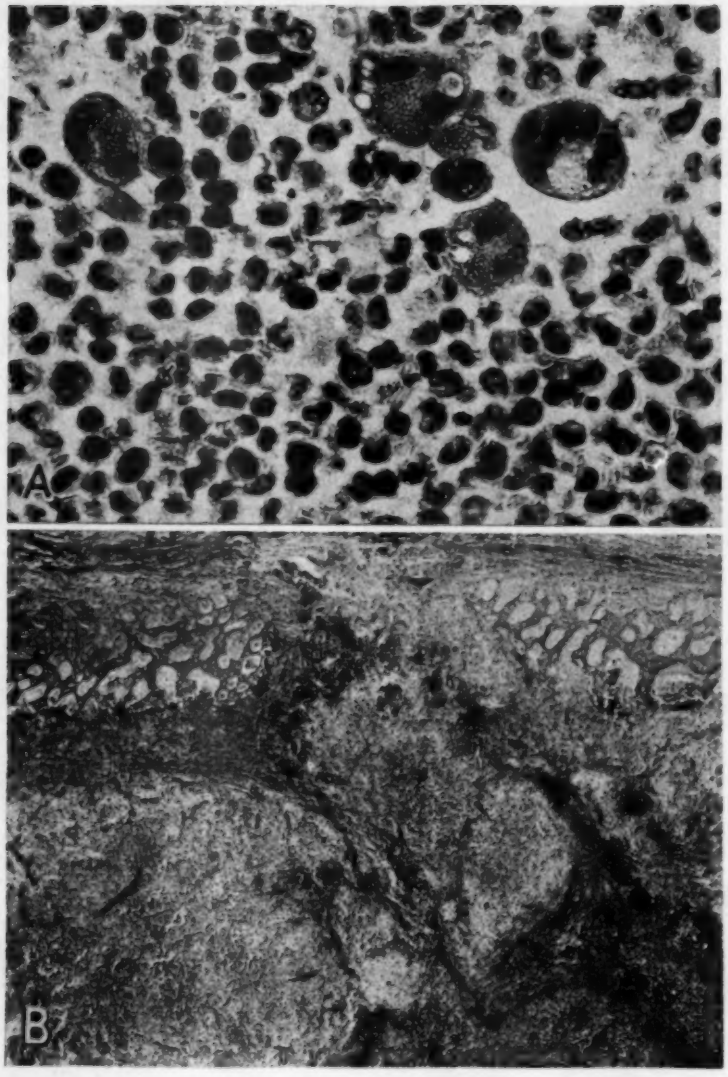

Fig. 3.—*A*, photomicrograph (× 270) showing a number of multinuclear giant cells in a field also containing many histiocytes. Some of the giant cells show vacuoles representing the sites of phagocytosed cells. The stippling of the cytoplasm of the giant cells results from the presence of brownish (presumably hemosiderin) pigment granules. The cytoplasm of many of the histiocytes also contains fine brownish granules. In other fields on this slide, from a lesion which was again the only one present in the case, eosinophils were prominent.

B, photomicrograph (× 15) of a rib which is the site of a lesion of eosinophilic granuloma of bone, in a case in which the disease was limited to this bone. The affected portion of the rib is the site of a pathologic fracture. Note that there is a defect in the cortex and that the diseased tissue in the interior of the rib appears partitioned off into fields. Study of the slide under higher magnification shows that the delimited areas consist mainly of histiocytes intermingled with larger or smaller numbers of eosinophils. The delimiting strands and tracks are composed of vascular connective tissue permeated by eosinophils. This permeation is often heavy, as on the under surface of the cortex on the left and in the lower right hand corner.

This conception may be correct, but it is unfortunate that the authors failed to document it with pertinent photomicrographs of untreated lesions in chronologic sequence to show the later changes in the direction of lipogranuloma and so on as described by them. In the following section, we shall describe two untreated lesions in a case of eosinophilic granuloma of bone which appear to indicate that such lesions may heal through resolution instead of through the mediacy of lipidization and connective tissue scarring.

Healing of the Untreated Lesion Apparently Through Resolution.—The two lesions studied which seem to indicate that healing may take place through resolution were in ribs.

The patient was the 8 year old child (a girl) previously mentioned in whom we were able to demonstrate roentgenographically eight lesions, only two of which (the one in the right femoral neck and the one in the body of the seventh cervical vertebra), as noted, had given rise to clinical difficulties, the other lesions being entirely silent clinically.

The illness began about Feb. 1, 1941, with moderate generalized abdominal pain which gradually subsided in the course of the succeeding week. When the patient was then allowed to be out of bed, she complained of a mild, barely perceptible pain in the right groin on walking, and walked with a limp on the right side. About two weeks after the onset of the illness, a roentgenogram of the hip in question revealed a rarefied area in the right femoral neck and also revealed incidentally a rarefied shadow in the upper part of the shaft of the left femur.

On admission to the hospital,[13] on March 18, the patient's entire skeleton was roentgenographed, and among the other lesions of bones found were those in the two ribs which concern us here. The posterior portion of the right ninth rib showed, several inches from its head, a rather large area which was modified through expansion and rarefaction. The corresponding portion of the right eleventh rib seemed also to have a large osteolytic destructive lesion. The shadow cast by the diaphragm and the liver made its visualization (and a fortiori its reproduction) difficult in the films taken on admission, but it was plain in the film taken two months later (fig. 4 *A*).

The surgical intervention undertaken three days after admission consisted of curettage of the neck of the right femur and of the upper portion of the shaft of the left femur. The granulation tissue curetted from both these lesions revealed histologically the picture typical of eosinophilic granuloma of bone in its acute stage. No other lesions of bones were explored and none of these lesions (including those which were curetted) received any roentgen therapy. We were particularly interested in following the course of the surgically treated and also that of the untreated lesions in the absence of roentgen therapy, for we already knew that curettage and supplementary treatment with roentgen rays led to healing of the lesions of eosinophilic granuloma of bone.

By means of four successive roentgenologic examinations made in the course of the next nine months, it could be demonstrated not only that the curetted femoral lesions were healing in and ossifying but that the two untreated lesions in the ribs were doing so also (fig. 4 *B*). On December 24 (nine months after the patient's original admission and ten and one-half months after the apparent onset of the illness) consent was obtained for resection of the involved areas of the ribs in question, so that it was possible to ascertain directly what had actually happened in the course of the spontaneous healing of the lesions. Subperiosteal resections were made, 5 cm. of each of the ninth and eleventh ribs being removed.

Both rib segments were cut up into small blocks, and almost all these blocks were serially sectioned and studied microscopically. Surprisingly, this study revealed but little evidence of previous disease in the interior of the ribs in any of the sections. It was clear from all the sections that the interior of the ribs now contained a substantial amount of hemopoietic and fatty marrow between reconstituted spongy trabeculae. However, this hemopoietic marrow showed an excess of leukocytes, especially of eosinophilic leukocytes, and in some places the excess of the eosinophils was even pronounced. Furthermore, one could still see in the marrow here and there some macrophages containing blood pigment and even phagocytosed red blood cells. The sections which were prepared from a transverse slice through the widest portion of the ninth rib showed the strongest evidence of residual disease (figs. 4 *C* and 5 *A*). However, even this consisted merely of one small field in which there were many histiocytes filled with blood pigment and fragmented red blood cells, supported in a loose and somewhat vascular and edematous meshwork. The latter was rather heavily infiltrated with small cells having dark-staining nuclei and appearing to be young marrow cells.

Altogether, then, such traces of residual disease as we could still find in the great number of tissue slides prepared from all parts of the two rib segments were at most reminiscent of the original inflammatory histiocytic and eosinophilic lesion. Even though one now found somewhere in these healed lesions a few nests of foam cells, this finding in itself would not be significant as pointing in the direction of lipogranuloma. Indeed, a few small nests of foam cells would have little specific meaning, since they are frequently encountered even in the inflammatory granulation tissue of chronic pyogenic osteomyelitis. Nevertheless, it may be countered that a lipogranulomatous process had replaced the histiocytic-eosinophilic process but had already disappeared by the time we examined the ribs microscopically. As noted, Green and Farber held that this is indeed the chronology of the natural healing of the eosinophilic granulomatous lesion. It may be so, but it seems to us that the two rib specimens now described failed to yield any evidence in that direction and furthermore suggested instead that the lesion of eosinophilic granuloma of bone can heal by resolution without passing through a lipogranulomatous stage.

13. Thurm, A. S.: Bull. Hosp. Joint Dis. **3**:9, 1942.

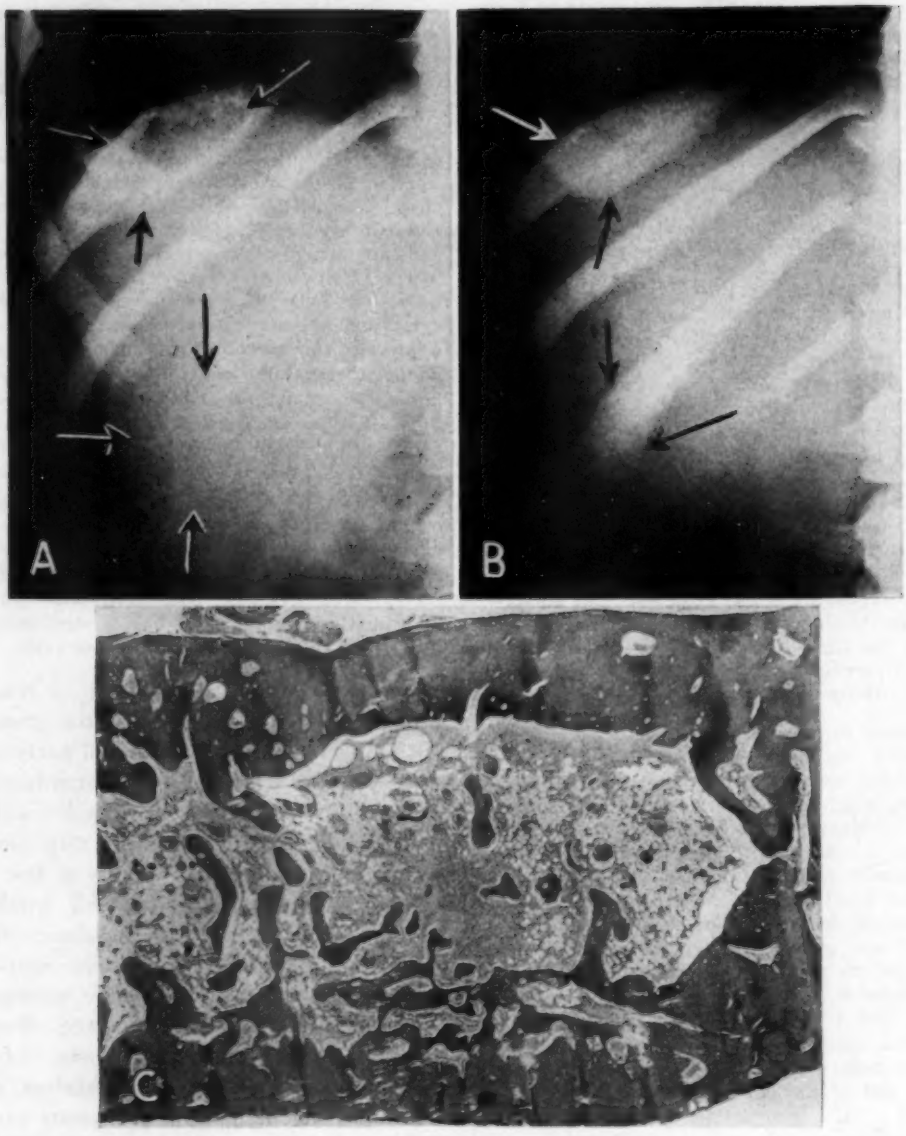

Fig. 4.—*A*, roentgenogram showing lesions of eosinophilic granuloma of bone involving the right ninth and eleventh ribs of the patient previously referred to in figure 1 *A*. These lesions had already been visualized in the admission roentgenogram, taken two months earlier, but the interfering shadow cast by the liver makes reproduction of that earlier film useless for demonstration purposes.

B, roentgenogram showing the ribs illustrated in *A* as they appeared seven months later. Note the healing (spontaneous) of the ninth rib and the even more conspicuous healing of the eleventh.

C, photomicrograph (× 15) of a tissue slide from the lesion of the ninth rib shown in *B*. The slide was prepared from a block of tissue taken through the widest part of the affected area of the rib. The interior of the rib shows a marrow space containing fatty and myeloid marrow and numerous dilated blood channels. No obvious scarring is present, and what little diseased tissue is visible at all is to be seen mainly in the middle of the picture. For details, see figure 5 *A*.

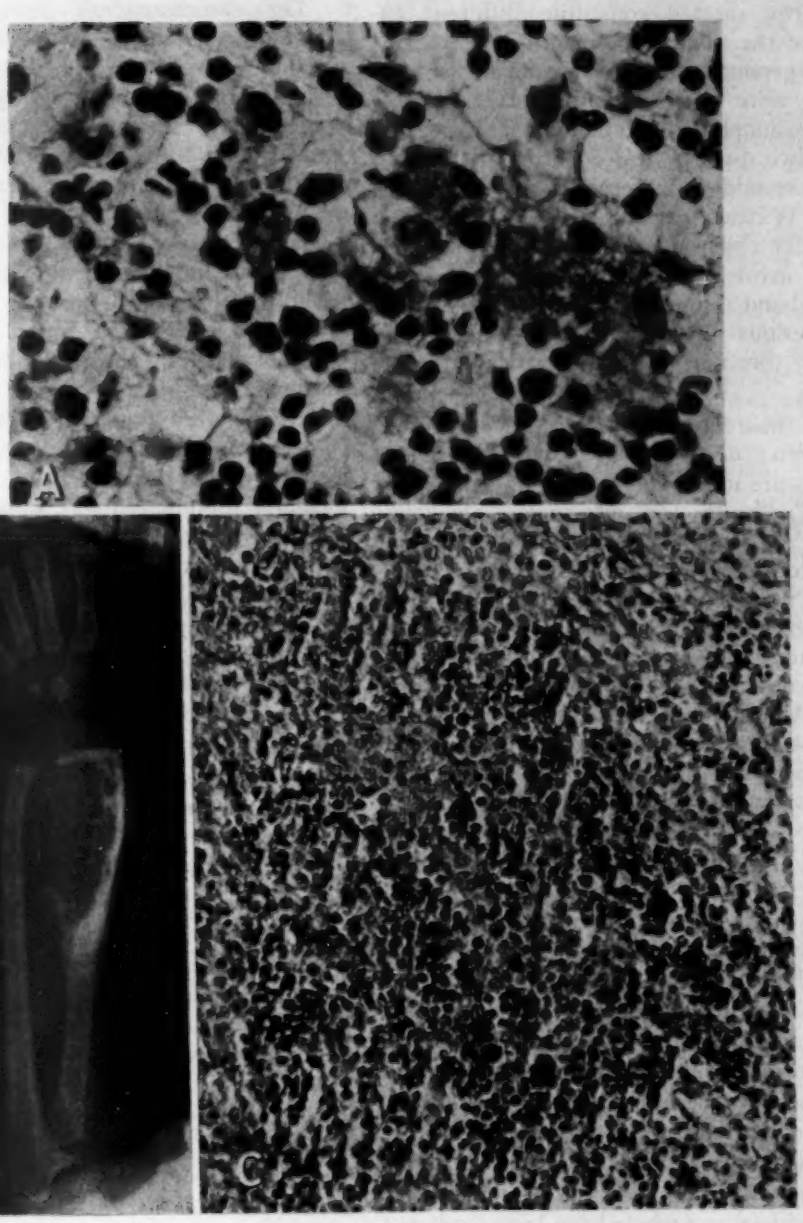

Fig. 5.—*A*, photomicrograph (× 475) showing cellular details in the diseased area illustrated in figure 4 *C*. Note the giant histiocytes, which are supported in a loose, edematous meshwork and contain pigment granules in their cytoplasm.

B, roentgenogram showing a destructive lesion in the radius of a girl 10 months old. This infant was suffering from Letterer-Siwe disease and died about two months after this roentgenogram was taken. Destructive skeletal lesions were not present in any of the other bones at the time when the lesion in the radius was discovered.

C, photomicrograph (× 150) of tissue curetted from the lesion illustrated in *B*. The lighter cells are histiocytes and the darker ones eosinophils. In respect to cytologic appearance the curettings from this lesion of bone in a case of Letterer-Siwe disease are interchangeable with those from a lesion in a case of eosinophilic granuloma even when the latter disease is limited to one bone.

LETTERER-SIWE DISEASE (RETICULOSIS, RETICULOENDOTHELIOSIS, NONLIPOID HISTIOCYTOSIS)

As already noted, it appears that eosinophilic granuloma of bone (conceived in a clinicoanatomic sense), Schüller-Christian disease and Letterer-Siwe disease constitute different expressions of the same basic disorder. Certainly, on clinical grounds alone one would not suspect that there were any points of correspondence between eosinophilic granuloma of bone and Letterer-Siwe disease. Indeed, from all that has already been said about eosinophilic granuloma of bone it is clear that the latter is clinically a mild disorder apparently limited to the skeleton and often involving only a single bone. On the other hand, Letterer-Siwe disease is clinically a serious disorder with lesions widely distributed through the body, including the skeleton.

Most of the relatively few recorded cases of "Letterer-Siwe disease" are to be found listed in the literature under such names as "aleukemic reticulosis," "acute (or infectious) aleukemic reticulosis," "reticulendotheliosis," "infectious granulomatous (or nonlipoid) reticuloendotheliosis" and "nonlipoid histiocytosis." Also, some cases are to be found reported in the literature dealing ostensibly with Schüller-Christian disease.[14] The designation "Letterer-Siwe's disease," coined by Abt and Denenholz [15] in 1936, has the obvious disadvantages inherent in naming disorders after persons. It seems to represent an attempt to give credit to Letterer [16] for his description of a case from the standpoint of pathology in 1924 and to Siwe [17] for his compilation of the clinical observations in 1933 on the basis of a case of his own and a number of cases already published by others. It should be noted, however, that Letterer held at first that the pathologic changes underlying the disorder have a tumorous basis, while the common current opinion is that they represent an inflammatory reaction, probably to an infection. Furthermore, the clinical picture as delineated by Siwe has been found to require supplementation and even amendment. Be that as it may, valuable contributions to understanding of the subject as a whole have been made not only by certain of the authors already mentioned [18] but also by Guizetti,[19] Podvinec and Terplan,[20] Roussy and Oberling,[21] Foot and Olcott,[22] Paige [23] and Van Creveld and Ter Poorten,[24] among others.

The clinicoanatomic complex of Letterer-Siwe disease makes its appearance mainly in infants and in children below the age of 2 (rarely appearing in those beyond the age of 4) and usually runs an acute or subacute course, fatal within a matter of weeks or months. In some cases, however, the course is semichronic, being protracted over a year or two or even a somewhat longer period. The condition may even pass over into what may already be called Schüller-Christian disease, as will be made clear presently.

The principal clinical findings presented by the cases as a whole are: a febrile course; enlargement of the spleen, of the liver and of superficial lymph nodes; a cutaneous eruption, generally purpuric in character and often particularly pronounced shortly before death; hypochromic secondary anemia, often profound terminally; one or more destructive lesions in bones, particularly commonly in the skull bones. Less regularly the cases present: leukocytosis of moderate severity; evidence of secondary bacterial infection in the form of purulent otitis media and mastoiditis, ulcerative or even necrotizing angina, acute lymphadenitis and other conditions; mottling or honeycombing and even emphysema of the lungs, evident roentgenographically. Terminally, pleural and abdominal effusion and subcutaneous edema may appear.

Anatomically, Letterer-Siwe disease is characterized by wide dispersion of histiocytes through the tissues of the body, in the form of either nodular foci or more diffuse collections. Histologic examination reveals that as more or less conspicuous nodular collections the histiocytes occur particularly in the lymph nodes, the tonsils, the thymus, the spleen, the bone marrow, the liver, the lymphoid tissue of the alimentary tract and the skin. Diffusely distributed they are found particularly in the lungs, the dura, the

14. If in the present light one studies the case which has linked the name of A. Hand (Arch. Pediat. **10**: 673, 1893; Am. J. M. Sc. **162**:509, 1921) with the names of Schüller and Christian, it seems more than likely that that case should be classed under Letterer-Siwe disease rather than under Schüller-Christian disease.

15. Abt, F., and Denenholz, E. J.: Am. J. Dis. Child. **51**:499, 1936.

16. Letterer, E.: Frankfurt. Ztschr. f. Path. **30**:377, 1924.

17. Siwe, S. A.: Ztschr. f. Kinderh. **55**:212, 1933.

18. See footnotes 6, 7, 8, 15, 16 and 17.

19. Guizetti, H.-U.: Virchows Arch. f. path. Anat. **282**:194, 1931.

20. Podvinec, E., and Terplan, K.: Arch. f. Kinderh. **93**:40, 1931.

21. Roussy, G., and Oberling, C.: Wien. med. Wchnschr. **84**:407, 1934.

22. Foot, N. C., and Olcott, C. T.: Am. J. Path. **10**: 81, 1934.

23. Paige, B. H.: Am. J. Dis. Child. **49**:266, 1935.

24. Van Creveld, S., and Ter Poorten, F. H.: Arch. Dis. Childhood **10**:125, 1935.

periosteum overlying affected bone areas, the interstitial or fatty connective tissue of the heart, the pancreas and the renal pelves and even in certain of the endocrine glands (pituitary, parathyroid and adrenal). Interspersed among the histiocytes there may be small numbers of lymphocytes and plasma cells and sometimes also a sprinkling of eosinophils.

A basis of relationship between Letterer-Siwe disease and eosinophilic granuloma of bone is afforded by the histoanatomic picture of the destructive skeletal lesions in the former. Specifically, the granulation tissue present in the destructive skeletal lesions of Letterer-Siwe disease in an early stage of their evolution may be indistinguishable microscopically from such tissue in corresponding lesions of eosinophilic granuloma of bone. A case mentioned in a footnote to our paper on eosinophilic granuloma of bone [25] turns out to be relevant here. This was the case of an infant presenting a large destructive lesion of a radial shaft from which material obtained in curettements showed the histologic picture of the lesion of eosinophilic granuloma of bone (fig. 5 *B* and *C*).

The patient was a 10 month old girl who was admitted to the hospital [26] primarily because of pain and swelling of three weeks' standing in the area of the left wrist and forearm. There was no history of antecedent trauma to the region in question. Roentgenologic examination of the skeleton revealed a lesion involving over one third of the left radial shaft at the distal end but no lesions in other bones.

However, the course of the disease in this infant was rapidly downhill during the subsequent two months. She was febrile and there was associated enlargement of the liver, the spleen and the superficial lymph nodes, as well as extensive alterations in the skin, including scaling of the scalp (resembling seborrheic dermatitis), superficial ulcerations and innumerable petechial hemorrhages. Thus, clinically there was evidence that the infant was suffering from Letterer-Siwe disease, and the observations at autopsy confirmed this evidence. Indeed, both the clinical and the anatomic findings in this case are in line with those in the cases reported in the literature under the headings of Letterer-Siwe disease, reticulosis (or reticuloendotheliosis), nonlipoid histiocytosis, and others, and reviewed by us in connection with the study of this case. Furthermore, the evaluation of the histologic changes in the case (fig. 6) was greatly aided by the opportunity of reviewing also, for comparison with it, the histologic slides in the relevant cases reported by Abt and Denenholz,[15] Paige [23] and Foot and Olcott.[22]

Sections prepared from the various organs and tissues removed at autopsy in the case described showed on microscopic examination a wide distribution of histiocytes through the body tissues, specifically in lymph nodes (both peripheral and internal) and in the spleen, the liver, the marrow, the lungs, the skin, the lymphoid tissue of the large intestine and the appendix, the periappendical fat tissue, the interstitial tissue of the pancreas, the peripancreatic (subperitoneal) connective tissue, the adrenal medulla, the periadrenal fat and the hili of the kidneys. Other changes included focal necroses in the spleen, severe degeneration and fatty change of the hepatic parenchyma, degeneration of the renal epithelium and of the myocardium and the presence of foci of extramedullary hemopoiesis in the liver and kidneys.

The collections of histiocytes in the lymph nodes, the intestinal lymphoid tissue and the spleen were particularly large, and focal nodular groups of them even tended to be confluent (fig. 6 *A* and *B*). In the other tissues, on the whole, the nests were relatively small and tended to be well separated, or the histiocytes were diffusely distributed (fig. 6 *D*). The histiocytes presented on the whole faintly acidophilic cytoplasm and rather large pale rounded or oval nuclei (fig. 6 *C*). Some of the cells were binuclear, and some rather large ones were even multinuclear. Many of the histiocytes contained phagocytosed red blood cells and brownish pigment granules in their cytoplasm. Some of the larger nodular collections presented evidence of hemorrhage and of degeneration or necrobiosis of their component cells and even some fibroblastic scarring. Furthermore, in some sites the focal collections of macrophages were interspersed with a few plasma cells, lymphocytes and polymorphonuclear leukocytes.

Thus the case of the infant just considered enables us to recognize a connection between Letterer-Siwe disease and eosinophilic granuloma of bone. This connection resides in the anatomic character of the lesion in the radius of the patient. As noted, when this lesion was examined, it presented a histologic picture identical with that seen in evolving lesions of eosinophilic granuloma of bone. That is, one saw hemorrhage and necrosis in the inflammatory tissue and large fields of histiocytes intermingled with fields of eosinophils—a picture which when once seen is sufficiently striking to remain firmly in one's mind (fig. 5 *C*). Why it is only in the destructive lesions of bones (and early in their evolution) that one finds the large numbers of eosinophils coloring the histiocytic granulation tissue and why, specifically, the lesions in the internal organs and lymph nodes in Letterer-Siwe disease fail to show this imprint of eosinophils are interesting and still unanswered questions. At any rate, that is what one finds.

On the other hand, in relatively old or more chronic skeletal lesions—that is, lesions in cases of Letterer-Siwe disease in which the disorder

25. Lichtenstein and Jaffe,[1] p. 600.

26. The infant was admitted originally to the Brooklyn Cancer Institute, in March 1940, and Dr. Herman Bolker, then pathologist there, placed at our disposal at that time the roentgenographic films of the left forearm and wrist, along with tissue obtained by curettements and slides. In May she was transferred to the King's County Hospital, where she died and an autopsy was made. Dr. Caspar Burn, of the department of pathology there, made available to us a clinical abstract, the autopsy protocol and tissue sections from this particularly instructive case.

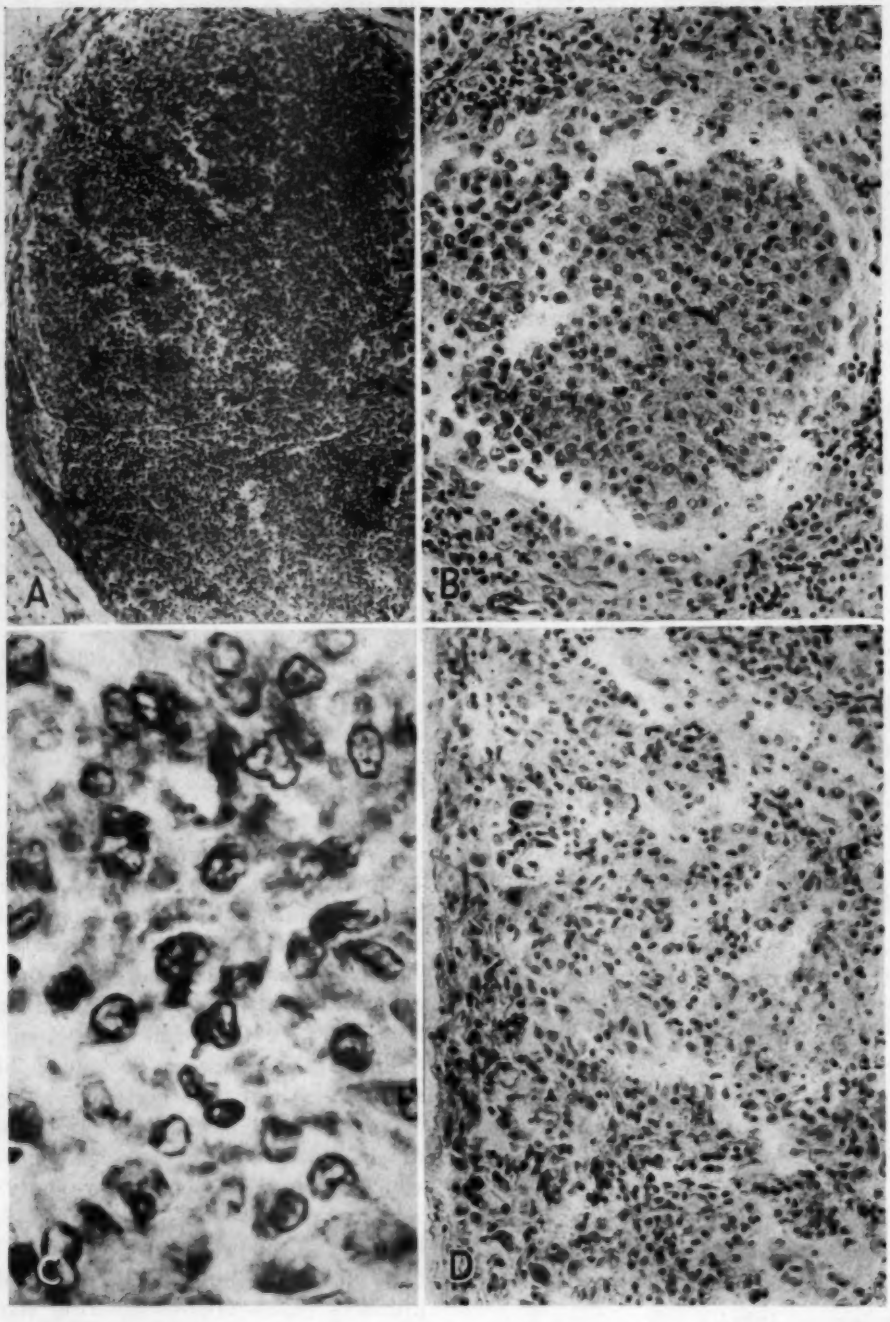

Fig. 6.—*A*, photomicrograph (× 60) of a lymph node removed at autopsy in the case of Letterer-Siwe disease mentioned in the legend for figure 5 *B*. Note the substantial replacement of the lymphoid tissue by cells which are histiocytes.

B, photomicrograph (× 125) showing a nodular focus of histiocytes in an affected lymph node in the case mentioned in *A*.

C, photomicrograph (× 400) showing the histiocytes of *B* under higher power.

D, photomicrograph (× 100) showing a pulmonary field containing numerous histiocytes in the case referred to under *A*, *B* and *C*.

has run for more than a year—one is likely to find considerable fibrous scarring of the inflammatory tissue and transformation of some or many of the histiocytes into lipophagic foam cells, in conformity with the picture of so-called lipogranulomatosis supposedly characteristic of Schüller-Christian disease. One finds this fibrous scarring and secondary lipidization (through the conversion of histiocytes into lipophagic foam cells) not only in connection with the lesions of bones and their coverings but also in the lesions of the thymus and the lungs.

Thus, at one stage or another a case of Letterer-Siwe disease (reticulosis, reticuloendotheliosis, nonlipoid histiocytosis) may show connections through some of its lesions not only with eosinophilic granuloma of bone but also with Schüller-Christian disease (lipogranulomatosis). Anatomic support for this idea was given by Flori and Parenti [6] in 1937 in their paper on "hyperplastic infectious reticuloendotheliosis with granulo-xanthomatous evolution (of the type of Hand-Schüller-Christian)." It was also maintained by Glanzmann,[7] who held that cases of so-called reticulo-endotheliosis (Letterer-Siwe disease) may pass over into so-called Schüller-Christian disease. More specifically, he held that Letterer-Siwe disease apparently represents the acute septic expression of the pathologic process which in more chronic form is represented by Schüller-Christian disease. A similar point of view was taken by Wallgren,[8] who stressed the importance of the time factor in the transition from one disease state into the other. Other exponents of the anatomic kinship between the two disorders include Gross and Jacox [27] and Mallory.[9]

In connection with the question of the mergence of Letterer-Siwe disease in its chronic stage into what may already be called Schüller-Christian disease, a few additional remarks on the skeletal lesions in the former may be in order. As already noted, skeletal lesions evident clinically or at least roentgenographically in Letterer-Siwe disease are particularly likely to include lesions in skull bones. Specifically, it is the calvarium that is most likely to show one or more lesions in one or several of its component bones. However, involvement of the base of the skull is by no means uncommon, and occasionally one or another of the facial bones has been found affected. When lesions are present in bones of the skull, one is likely to find them elsewhere in the skeleton also, in one or even many bones, with apparent exception of the bones of the hands and feet. In the cases described by Gerstel [29] and by Flori and Parenti [6] the skeleton was particularly heavily affected.

Even when a number of bones (including bones of the skull) are involved, all of the skeletal lesions may be clinically silent. On the other hand, one of them may direct attention to involvement of the skeleton in general, so that others are then discovered roentgenographically. In connection with involvement of the sphenoid bone and of the bones delimiting the orbits, diabetes insipidus and exophthalmos have been observed. Indeed, some of the cases have even presented the complete Christian triad of calvarial defects, diabetes insipidus and exophthalmos.

SCHÜLLER-CHRISTIAN DISEASE (LIPOGRANULOMATOSIS)

As we shall show, the clinical category of Schüller-Christian disease is a miscellaneous one. This is true even apart from the fact, now clearly recognized, that the presence or the absence of the triad of calvarial defects, exophthalmos and diabetes insipidus is not a sound basis for decision as to whether or not a case belongs in the category of Schüller-Christian disease. As to the Christian triad, its presence depends solely on extensive involvement of the skull bones and is neither essential in the diagnosis nor specific for the disease.

However, it does appear that any case which is placed in the category of Schüller-Christian disease should be expected to show, as the anatomic prerequisite, collagenized and lipidized lesions classifiable as lipogranuloma (fig. 7). Nevertheless, one can often demonstrate in the lipogranulomatous lesions of a given case (much more readily in certain cases than in others) that the collagenization and lipidization has been engrafted on lesions which originally were dominated by the presence of compacted histiocytes still free of lipid. In other words, it seems to be fairly clear that the fibroblastic scarring and collagenization and the varying amounts of cholesterol (free or in the cytoplasm of lipophagic foam cells) which give the characteristic lipogranuloma imprint to the lesions of Schüller-Christian disease represent secondary engraftments on an inflammatory lesion originally neither scarred nor lipidized.

The question which now arises is: In Schüller-Christian disease, what is the cytologic character

27. Gross, P., and Jacox, H. W.: Am. J. M. Sc. **203**:673, 1942.

28. Footnote deleted by the authors.

29. Gerstel, G.: Virchows Arch. f.-path. Anat. **294**: 278, 1934.

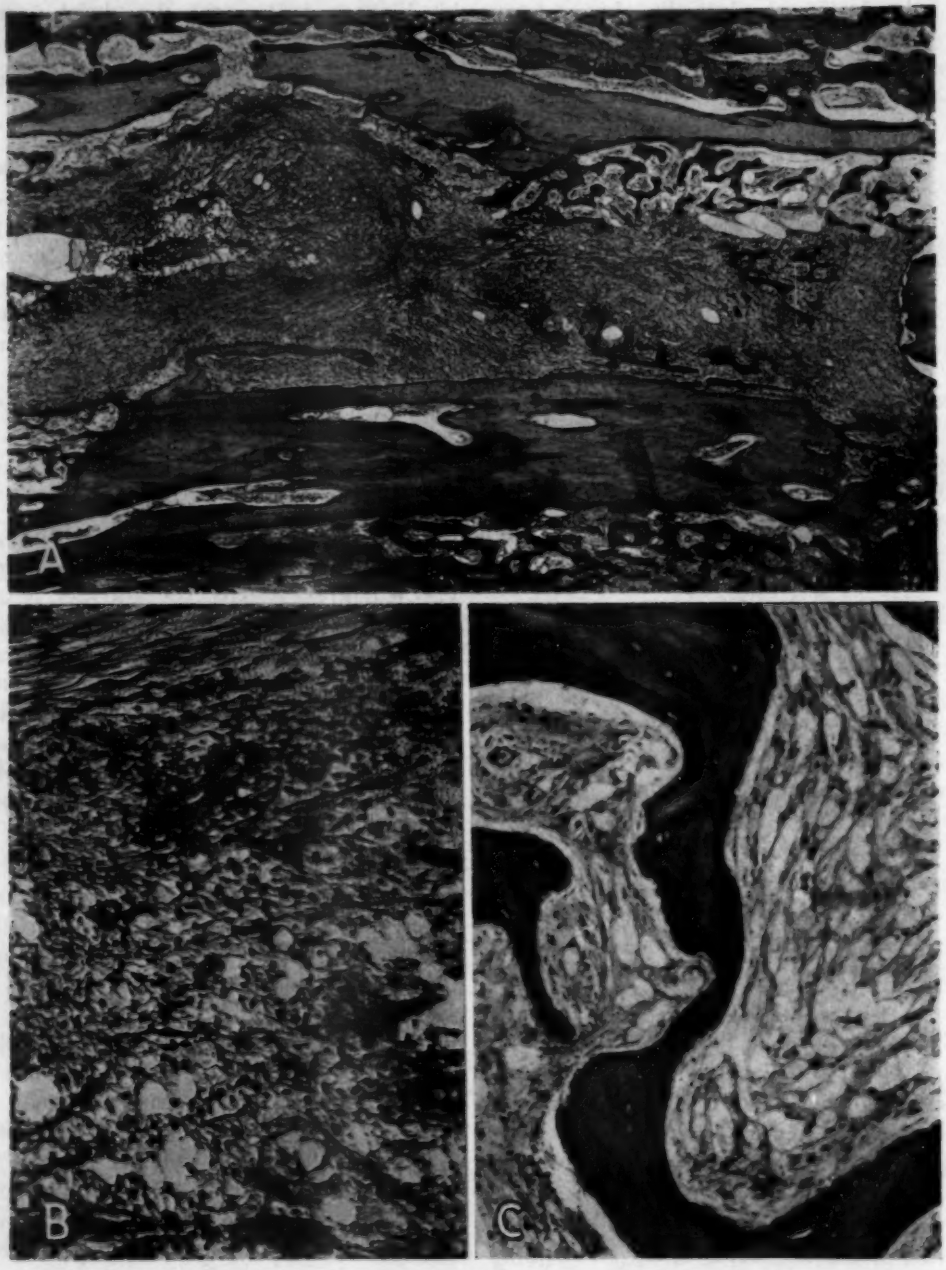

Fig. 7.—*A*, photomicrograph (× 16) showing the lipogranulomatous character of a lesion of a rib in a case of Schüller-Christian disease. The patient was a woman of 29 in whom the disease so far as the skeleton was concerned seemed to be limited to the region of the left sacroiliac joint and to portions of the right seventh and eighth ribs. Clinically, this was an ambiguous case of Schüller-Christian disease, the diagnosis of which was made only after biopsy. Note the fracture, the periosteal apposition of new bone and the alteration in the interior of the bone.

B, photomicrograph (× 225) showing lipoid-containing (foam) cells in the delimited roundish focus in the upper central part of *A*.

C, photomicrograph (× 100) showing the lipogranulomatous character of the bone lesions in the case of a woman of 44 with Schüller-Christian disease. In addition to lesions in the skeleton, she also showed lipogranulomatous lesions in the lungs, in the heart and to an insignificant degree in the liver. The scarring of the lungs in this case was so extensive that it had led to cardiac hypertrophy and failure of the right side of the heart.

of the skeletal lesions in particular before they undergo scarring and lipidization? That is, do they show, before that, a granulation tissue dominated by the presence of histiocytes or of histiocytes and eosinophils—a picture such as one sees in the lesions of eosinophilic granuloma of bone? The 3 cases of Schüller-Christian disease on which we ourselves have anatomic material (biopsy in 1 and autopsy in 2) do not yield an answer to this question since the skeletal lesions studied in these cases were all in the lipogranulomatous stage when they were examined.

Nevertheless, it is logically probable that the lesions of bones in Schüller-Christian disease may originally resemble those of eosinophilic granuloma of bone. This assumption is based on the fact that, on the one hand, in the early stages of evolution of Letterer-Siwe disease one may find destructive skeletal lesions resembling cytologically those of eosinophilic granuloma of bone, and, on the other hand, in its chronic stages Letterer-Siwe disease takes on the aspect of Schüller-Christian disease, the skeletal and even certain visceral lesions undergoing lipogranulomatous transformation. However, in our opinion, no case, irrespective of the clinical picture it presents, should be designated as one of Schüller-Christian disease unless or until at least some of its lesions (visceral or skeletal) already have the cytologic character of lipogranuloma.

How various are the clinical cases which one can put into the category of Schüller-Christian disease is already evident from some of our previous remarks in relation to Letterer-Siwe disease. In that connection it was stated that cases of Letterer-Siwe disease running an exceptionally protracted course of a year or several years may eventually cross the border into the domain of Schüller-Christian disease. The case reported by Merritt and Paige [30] seems to represent an excellent example of progression of the basic pathologic changes from those of Letterer-Siwe to those of Schüller-Christian disease.

Dr. Beryl Paige, pathologist to the Babies Hospital in New York, turned over to us the tissue slides in that case and demonstrated a steady interest in the various questions involved.

The subject in that case was a child who died at the age of 5, after being ill for two years, and who presented in the course of the protracted illness not only fever and a cutaneous rash, along with enlargement of the lymph nodes, of the spleen and of the liver, but also the complete Christian triad. The spleen, the liver and the lymph nodes showed focal nodular collections of histiocytes such as one sees in infants presenting the Letterer-Siwe syndrome in the typical acute form. However, in the skull bones (and particularly in the orbital bones, the sphenoid and the temporal and parietal bones) the lesions showed fields of lipophages in a meshwork of fibroblasts and collagen fibers. These fields alternated with fields of histiocytes (some spindle shaped) which as yet had not taken up lipid. The dura in the area of the involvement of the skull bones was irregularly thickened in some places, and the thickened portions presented a yellowish, rubbery, fibrous appearance. Microscopically, such altered dura was found composed of collagenous connective tissue heavily interspersed with histiocytes which on the whole tended to show foamy cytoplasm. In addition, it contained many bizarre giant multinuclear cells whose cytoplasm was likewise foamy. Furthermore, the thymus in this case showed striking changes in the direction of lipogranulomatosis, as did the lungs and one of the parathyroid glands.

Such cases, with their coloring of Letterer-Siwe disease, relatively rapid course and appearance in very young children, contrast with the classic cases of Schüller-Christian disease. In the latter cases (of which the one reported by Chiari [31] is a good example), even though the subjects come to present the full Christian triad, the course of the illness is more protracted, sometimes extending over a period of ten or even fifteen years. Furthermore, while the illness is often found to have set in at some time between the ages of 5 and 10, there are many cases in which the onset dates from some time in the second or third decade or even later.

One or more phenomena of the Christian triad may first direct attention to the presence of the disease. On the other hand, in some young subjects it may be stunting of growth that draws notice to the illness. In other subjects the first abnormality observed may be a lesion in a jaw, in a long bone or even in some other bone. In association with these various skeletal lesions and some or all of the features of the Christian triad one may find pulmonary fibrosis, adiposity and even hypogenitalism. Altogether, there is a wide variety of possible findings in the classic cases of Schüller-Christian disease.

As to the ultimate prognosis, this is serious if, on account of lesions in the base of the skull, there has been damage to the hypothalamus, the infundibulum and the pituitary gland or if, on account of lesions in the lungs, the latter have undergone scarification leading to failure of the right side of the heart. Even the heart and the brain and cord or their coverings may be the site of lesions inimical to life.

Juxtaposed against such cases are those in which the clinical picture hardly suggests Schüller-Christian disease at all, and it seems useful to give a few details on a case in point.[32]

30. Merritt, K. K., and Paige, B. H.: Am. J. Dis. Child. **46**:1368, 1933.

31. Chiari, H.: Ergebn. d. allg. Path. u. path. Anat. **24**:396, 1931.

32. Sonnenschein, H. D.: Bull. Hosp. Joint Dis. **2**: 63, 1941.

The patient was a woman of 29 who, a few weeks after delivery of a 7½ month fetus, noticed pain across the lower part of the back and particularly in the left sacroiliac region. Roentgenograms of the skeleton taken some weeks after the appearance of this pain showed a large destructive process about the left sacroiliac articulation and destructive changes involving the right seventh and eighth ribs. The skull bones presented nothing abnormal. The nature of the whole clinical picture in this case was such as to have led to interpretation of the skeletal abnormalities as manifestations of carcinoma metastatic to the bones from an undetermined focus. In a desire to obtain further information, a biopsy specimen was removed from the affected right seventh rib. Study showed that the lesion there was clearly lipogranuloma (fig. 7 *A* and *B*). The patient received 1,200 roentgens to the sacroiliac region and ribs. The lesions healed rapidly, and the patient has had no further skeletal difficulties in the ensuing seven years.

In connection with such clinically ambiguous cases, one may mention 2 (with autopsy observations) reported by Chester.[33] (The material from these 2 cases was available to us for restudy, since Chester's report emanated from the laboratory of the late Viennese pathologist, Jacob Erdheim, and much of the latter's private collection of interesting pathologic material is now housed in our laboratory.) One subject was a woman of 44 and the other a man of 69, and in neither case was lipogranulomatosis suspected clinically, though in both cases the presence of xanthelasmic patches on the eyelids was noted at autopsy. Both subjects were free of skeletal complaints during life, but the presence of skeletal lipogranulomatosis (particularly in the diaphyses of the long bones) was discovered when bones removed routinely at autopsy were cut open (fig. 7 *C*). Furthermore, in the woman, lipogranulomatous lesions were found in the lungs, in the heart and to an insignificant degree in the liver. The scarring of the lungs in this case was so extensive that it had led to cardiac hypertrophy and failure of the right side of the heart. Indeed, in some pulmonary fields the fibrous replacement was so thorough that the damaged parenchyma could scarcely be recognized as such. In addition, the lipogranulomatous lesions in the bones had provoked so pronounced an osteoplastic reaction there that the affected areas were extremely sclerotic.

TREATMENT OF EOSINOPHILIC GRANULOMA OF BONE

Patients with eosinophilic granuloma of bone involving a single bone come to the hospital only because of local complaints referable to the affected area of the bone and usually come under surgical treatment rather promptly, with one diagnosis or another. Since the definitive diagnosis is ordinarily made only after histologic study of tissue obtained from the lesion, one cannot know what would regularly happen to any solitary lesion if it were left undisturbed. We do know, however, that solitary lesions which are curetted show gradual repair of the affected area after this procedure alone. More often, curettage and supplementary radiation therapy (with employment of relatively small doses) have been used, but it is not certain that the roentgen therapy accelerates the healing process.

Roentgen therapy alone has also been used against the remaining lesions in cases of multiple involvement of bones after the diagnosis has been made on the basis of curettings from one of the lesions. For such cases Green and Farber seem to advocate giving a total of 1,500 r through two fields, in divided doses, over a period of six days. Apparently, however, under such treatment, while some lesions heal within a few months, others do not, requiring a year or more.

However, we do know from our case with lesions of the ribs, already discussed, that in cases with multiple involvement of bones the lesions of the bones may undergo spontaneous healing. Specifically, in that case we were able to prove by anatomic examination of segments taken from two ribs at the sites of previous florid lesions that practically complete spontaneous healing by resolution had occurred in the course of seven months.

COMMENT AND CONCLUSIONS

The condition of eosinophilic granuloma of bone has been presented as a clinicoanatomic concept. In this condition the pathologic changes seem to be limited to the skeleton, and in any given case only one bone or, on the other hand, several or even many bones may be involved. A skeletal lesion of only weeks' or months' standing (and not modified by a fracture thorugh it) appears grossly as a more or less hemorrhagic and cystic area from which only a relatively small amount of granulation tissue can be curetted. This tissue where it is not necrotic is found to consist histologically, in its essence, of conspicuous numbers of histiocytes, including some revealing phagocytic activity, interspersed among which are more or less prominent accumulations of eosinophils, especially of eosinophilic leukocytes.

Thus, in principle there is little if any scarring, but if a fracture through the site of the lesion has been followed by a reparative reaction to it, considerable scarring may be manifest. Indeed, the interior of the bone at the site of the lesion

33. Chester, W.: Virchows Arch. f. path. Anat. **279**:561, 1930.

may then be found filled in more or less completely by a cellular and on the whole reddish-gray-white tissue. Histologically, one can still see in such a scarred lesion large fields presenting the characteristic picture of a granulation tissue dominated by histiocytes which are intermingled with eosinophils.

On the manner of healing of the untreated lesion of eosinophilic granuloma of bone there is as yet not complete agreement. Some hold that the lesion heals through the mediacy of connective tissue scarring and lipogranulomatous transformation and that after this the part undergoes anatomic restitution to a normal state. Our own experience does not include any anatomic material to sustain this conception, and we wish to point out that one should not deduce from the presence of scarring of the lesion in association with a fracture that scarring necessarily represents a phase in the natural healing of the eosinophilic granuloma as such. On the other hand, we have presented what we believe to be evidence that the untreated lesion can heal through resolution.

In any event, the condition of eosinophilic granuloma of bone offers an excellent prognosis. In cases with a solitary lesion, curettage of the lesion is followed by rather prompt filling in of the affected area of bone even without the use of supplementary roentgen therapy. In cases with multiple lesions, after the diagnosis has been made on the basis of a study of curettings from one of them, roentgen therapy may be given for the remaining ones. However, as has been indicated, lesions of eosinophilic granuloma of bone can heal without the benefit of either surgical curettage or radiation therapy.

Collaterally to the delineation of eosinophilic granuloma of bone as a clinicoanatomic concept, emphasis is laid on the intimate interrelations between this condition, Letterer-Siwe disease and Schüller-Christian disease. The three conditions constitute related expressions of the same basic disorder, whose lesions are characterized cytologically at their start by the presence in them of large numbers of histiocytes. That is, the basic anatomic disorder underlying the three conditions appears to be a peculiar inflammatory histiocytosis. As to its cause, the hypothesis which seems the most reasonable is that it develops in response to some as yet unknown infectious agent.

Eosinophilic granuloma of bone represents the mildest and most localized expression of the disorder. It may be that those in whom this form develops are those who have been able to overcome the disease process rapidly. There can be no doubt that the bone marrow is particularly susceptible to the causative agent of the disease, whatever the agent may be. Hence, the limitation of the disorder to one or more bones can plausibly be interpreted as representing successful localization of the disease to the skeleton.

Letterer-Siwe disease (reticulosis, reticuloendotheliosis, nonlipoid histiocytosis, and other names) represents the gravest and most generalized expression of the basic disorder. In it the histiocytic lesions are widely distributed, both through the soft tissues and the skeleton, but in their evolving stage the destructive lesions in the skeleton may show, like the lesions of eosinophilic granuloma of bone, an abundance of eosinophils intermingled with the histiocytes. Letterer-Siwe disease usually appears in infants or very young children. It is usually fatal within a matter of months, though occasionally it runs a more chronic course of a year or two or even more. In such instances, some of the lesions may become scarified and lipidized, so that the case eventually crosses the border into Schüller-Christian disease.

However, in most cases Schüller-Christian disease has a much more insidious evolution and runs a much milder and more chronic course. Indeed, the clinical category of Schüller-Christian disease is a miscellaneous one. It includes not only the severe forms which may be linked to Letterer-Siwe disease but also the classic ones presenting heavy involvement of the skeleton and showing the Christian triad in consequence of involvement of the skull in particular. In addition, it includes ambiguous forms which hardly suggest classic Schüller-Christian disease clinically, at all. It does appear that any case which is placed in the category of Schüller-Christian disease should be expected to show, as the anatomic prerequisite, already collagenized and lipidized lesions classifiable as lipogranuloma. However, one can often demonstrate, in the lipogranulomatous lesions of a given case (much more readily in certain cases than in others) that the scarification and lipidization have been engrafted on lesions which originally were dominated by the presence of compacted histiocytes still free of lipid.

SUMMARY

Eosinophilic granuloma of bone, Letterer-Siwe disease and Schüller-Christian disease may be considered as different clinicoanatomic expressions of the same basic disorder. This disorder apparently represents a peculiar inflammatory reaction to some as yet unknown agent of infection, and its individual lesions are characterized cytologically by the presence in them, at least at first, of large numbers of histiocytes. In eosinophilic granuloma of bone the lesions seem to develop

only in the skeleton and often only in a single bone, and are given a distinctive cytologic imprint by the abundance of eosinophils (especially eosinophilic leukocytes) interminged with the histiocytes. The lesions do not tend to become scarified, collagenized and lipidized, heal readily after simple curettage even without supplementary roentgen irradiation of the site, and may indeed heal by resolution even without any therapeutic intervention whatever.

In Letterer-Siwe disease the histiocytic lesions are widely distributed through the soft tissues (especially the lymphoid tissues) and the skeleton, the marrow sometimes being extensively affected even when there are few actually destructive lesions in the bones. With the presence of widespread destructive lesions in the bones of the skull, Letterer-Siwe disease may even come to present the complete Christian triad of calvarial defects, diabetes insipidus and exophthalmos. Though the lesions in the soft tissues in cases of Letterer-Siwe disease do not show infiltration by eosinophils, the destructive lesions of bones do show it, especially early in their evolution. Indeed, the granulation tissue in such destructive skeletal lesions of Letterer-Siwe disease is indistinguishable microscopically from such tissue in corresponding lesions of eosinophilic granuloma of bone. In a case of Letterer-Siwe disease running a relatively protracted course of a year or more one is likely to find that some of the lesions (especially those in the skeleton and those in the thymus and the lungs) are becoming scarified, collagenized and lipidized, the case thus passing over into the domain of Schüller-Christian disease.

Schüller-Christian disease represents the basic disorder in its most chronic and on the whole most miscellaneous form, the presence or the absence of the Christian triad depending merely on whether or not the skull has been heavily involved. However, any case which is placed in this category should be expected to show, as the anatomic prerequisite, at least some lesions which have already become scarified, collagenized and lipidized and hence classifiable as lipogranuloma.

NATURE AND OCCURRENCE OF THE CAVUM SEPTI PELLUCIDI

ORVAR SWENSON, M.D.

BOSTON

The cavum septi pellucidi has been described in anatomic works for over a century. In the protocols of routine autopsy material at the Children's Hospital in Boston the cavum has been described frequently, and it is often of such size that clinicians observing the sectioning of the brain ask the name and the significance of the space in the septum pellucidum. The cavum septi pellucidi is located in the midline of the brain within the septum pellucidum; it is bounded laterally by the leaves of the septum pellucidum, anteriorly by the genu of the corpus callosum, posteriorly by the anterior limbs and pillars of the fornix and inferiorly by the rostrum of the corpus and the anterior commissure. A second space in the septum pellucidum was described by Verga and is known as the cavum Vergae. It is located posterior to the cavum septi pellucidi, and in the material at the Children's Hospital, although rarely seen, it communicated with the cavum.

PRESENT STUDIES

My investigations were directed along three lines—to study the cavum in human brains from the standpoint of embryology, to record the incidence of the structure in the brains of children and adults, and to determine if there was any consistent anatomic connection of the cavum with the ventricular system.

The material for the study of the development of the cavum septi pellucidi in fetal human brains was secured from the collection of the late Dr. F. Tilney of the Neurological Institute, New York, through the assistance of Dr. Robert Loeb. This material consisted of progressive coronal sections of seven fetal brains from 2 months to 7 months old. The sections of the 2 month old fetal brain showed the septum pellucidum as a solid structure consisting of rather loose tissue without nerves. In the 3½ month old fetus a cavum was present, triangular in shape with a fairly broad base against the corpus callosum, which was quite well developed. Another significant change in this set of sections compared with the 2 month old brain was the development of the cortex, which had changed from several simple layers of cells in the younger specimen to a markedly thickened layer in the 3½ month old fetus. There are two layers of cerebral cortex in the median longitudinal fissure, and as they grow in thickness the two hemispheres are forced to become more separated. As the corpus callosum connects the two hemispheres, this separation must produce a stretching of the corpus callosum. This lateral force acting on the corpus callosum is transmitted to the septum pellucidum and causes the loose tissue to open and form the cavum. In all of the older fetal brains the cavum was present, triangular in shape with one side against the corpus callosum.

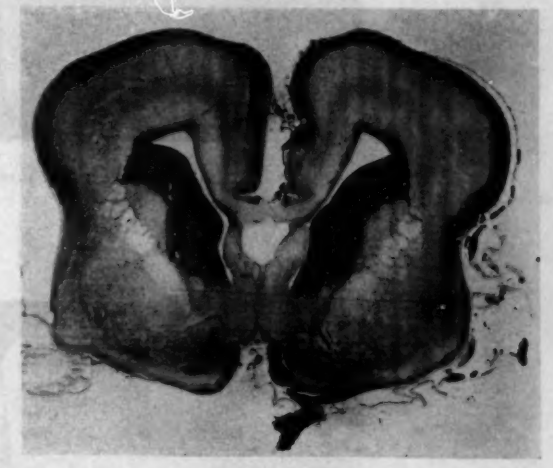

Fig. 1.—Coronal section of a 3½ month fetal brain containing a cavum septi pellucidi.

To establish the incidence of the cavum septi pellucidi in the brains of children and adults a careful study was made of autopsy material from the Children's Hospital and the Peter Bent Brigham Hospital. In looking for the cavum in human brains, care must be exercised or the structure will escape detection. If a brain is cut coronally at 2 or 3 cm. intervals, the cavum may be encased in a single section. To prevent overlooking the structure a special technic was established which consisted in making coronal sections until the tips of the lateral ventricles were encountered. A section about 3 cm. thick was cut, and a block of tissue including the septum pellucidum and corpus callosum was

From the departments of pathology of the Children's Hospital and the Harvard Medical School.

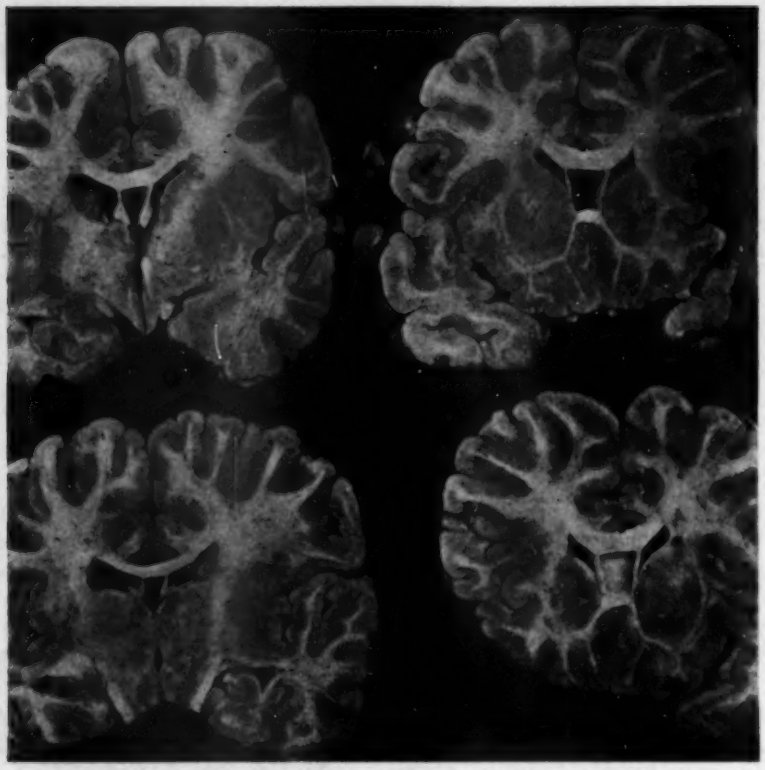

Fig. 2.—Coronal section of a child's brain with a cavum septi pellucidi which is definitely larger than the average seen in children.

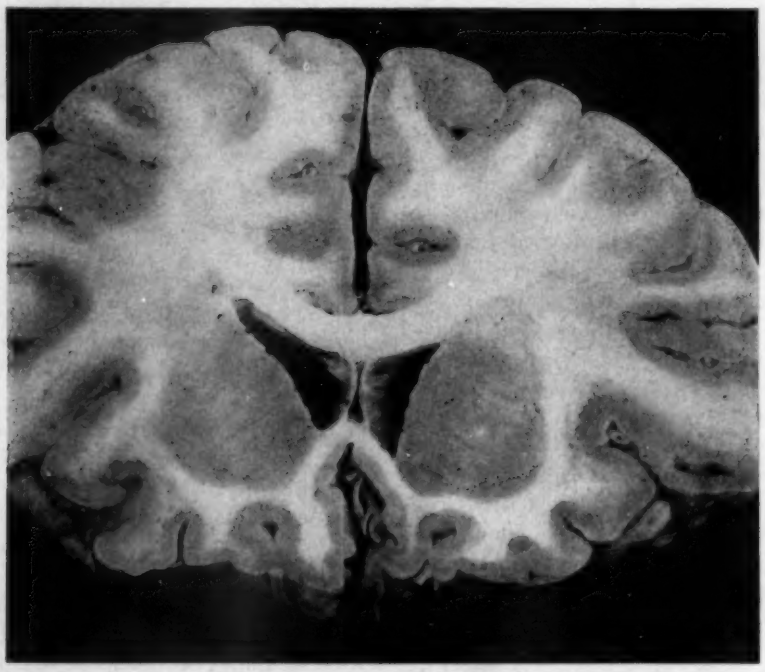

Fig. 3.—Coronal section of an adult brain with a cavum septi pellucidi of the type usually seen in adults.

removed and cut into sections 2 to 3 mm. in thickness. The value of this method is demonstrated by the fact that in 111 consecutive autopsies on children with the brain cut in a routine manner, a cavum was noted in only 34 brains,[1] while by the special technic it was demonstrated in 27 of 50 brains. In premature and young infants the cavum was observed to be usually the same shape as in fetuses, i. e., an almost equilateral triangular space with the base against the inferior surface of the corpus callosum. The size of the space varied greatly, averaging 1 cm. at the point of greatest width and 1.2 cm. in the greatest inferior-superior diameter.

A cavum septi pellucidi was recorded in the protocol in only 1 of 114 consecutive autopsies on adults. Of 50 adult brains examined by the special technic, 21 contained a cavum. In these brains the cavum was uniformly smaller than in the brains of children. The space averaged 0.6 cm. in the greatest superior-inferior diameter and 0.3 cm. in the greatest width. The decrease in size and the tendency to decrease in incidence indicate that the space gradually becomes obliterated as growth takes place. Histologic sections demonstrated a collapse with a filling in of the narrow space by glial proliferation.

In order to establish the presence of any anatomic connection between the cavum and the ventricular system, six specimens from essentially normal brains of various ages were removed in toto. Progressive sections were made, and 1 in 5 was mounted and stained. These were carefully studied for any anatomic communication with the ventricular system, and none was found. Parts of the walls of the cavum were lined with cells superficially like ependymal cells. They were mostly cuboidal, and at no time could cilia be identified in relation to these cells. From the material examined in this study it could not be definitely said that these cells were of ependymal or glial origin.

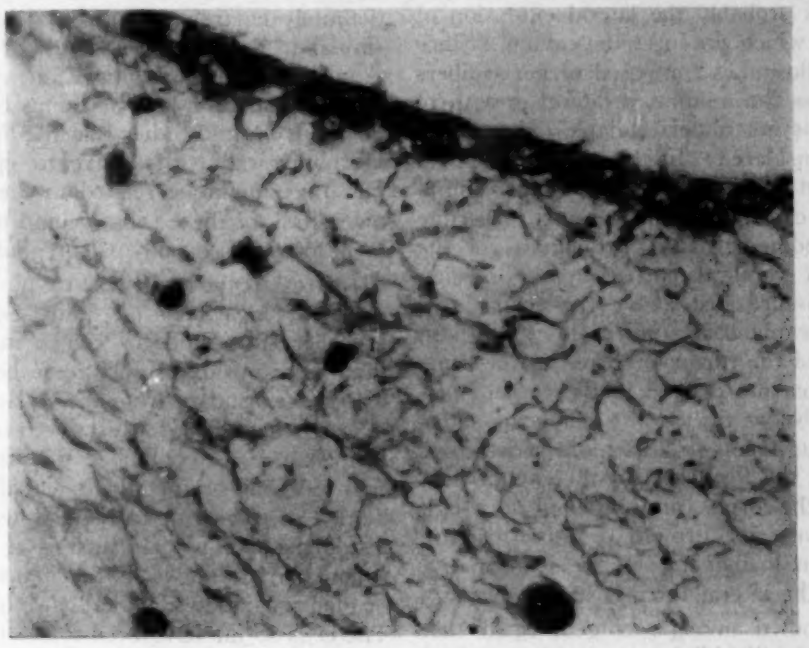

Fig. 4.—Photomicrograph showing cells lining parts of a cavum septi pellucidi.

COMMENT

There is a difference of opinion as to the origin of the cavum septi pellucidi. In some species the space may be related to incomplete fusion in the midline of the rudimentary brain. The best evidence in human brains suggests that there is a concrescence of the structures in the midline which becomes the stria terminalis. At a later period in the fetal development an opening appears in the stria terminalis which is the cavum septi pellucidi. My observations support this explanation of events in the formation of the cavum. In progressive coronal sections of a 2 month old fetal brain the septum pellucidum was a solid structure; yet in 6 fetal brains 3½ months of age and older the cavum was present. Thompson,[2] who studied the cavum septi pellucidi in several specimens of animals, expressed the belief that the best evidence presented so far

1. This analysis was made by Dr. Douglas H. Robinson in the laboratory of the Children's Hospital subsequent to the studies reported in this paper.

2. Thompson, I. M.: J. Anat. **67**:59, 1932.

supports the thesis that the cavum is formed by interstitial cleavage in the lamina terminalis. As to the actual formation of the cavum, Smith [3] expressed the belief that the growth of the dorsal commissure produces a stretching effect and forms the cavum in the septum pellucidum. It is difficult to see how the growth of the corpus callosum in a plane parallel to the long axis of the septum causes a space to open. From a mechanical standpoint it would seem that the important factor is a lateral force. Inasmuch as the cavum is most frequently found and is largest just posterior to the genu of the corpus callosum, it is probably the lateral extension of this structure which produces the cavum. Since the corpus callosum is composed of nerve fibers coursing across the midline, a lateral growth of the structure is not understandable unless other factors are considered. In this study of human fetal brains it was observed that the lateral stretch of the corpus callosum seemed to be due to the tremendous growth of thickness of the two layers of cerebral cortex in the median longitudinal fissure just above the corpus callosum. In the material studied this growth in the thickness of the cortex occurred at the same time that the cavum opened in the septum pellucidum just below the corpus callosum. This course of events seems to be a more satisfactory explanation of the formation of the cavum than the growth of the corpus callosum alone, although the anterior-posterior growth of this structure does play a part in that it holds the septum on a stretch while the lateral force is in action.

It is well known among roentgenologists that the cavum septi pellucidi is occasionally outlined after lumbar encephalograms have been made, and in these instances there undoubtedly is a communication with the ventricular system. The incidence in which this particular structure is noted during encephalography is far below the incidence which was found in my examination of autopsy material. Pendergrass and Hodes [4] noted the structure in 3 of 500 encephalograms. At the Peter Bent Brigham Hospital the cavum was reported seen once in 31 consecutive encephalograms and ventriculograms. This seems to indicate that in only a small percentage of brains does the cavum communicate with the ventricular system and that such communication is an abnormal condition. In the few cases in which there has been marked enlargement of the cavum, the lateral walls of the structure were often fenestrated so that there was free communication between the space and the ventricles, and this is probably the route by which air reaches the cavum. As far as I could tell from progressive sections, there is no anatomic communication with the ventricular system.

Wolf and Bamford [5] studied progressive sections of 1 definitely pathologically enlarged cavum and 4 small cavums. They found that fragments of the cavum were lined with low columnar and cuboidal cells which superficially seemed similar to ependymal cells. They expressed the opinion that these were not definitely ependymal cells as no blepharoplasts could be demonstrated in them. Kautzky [6] examined 5 definitely enlarged cavums and in 4 found cells similar to those observed by Wolf and Bamford. Among 16 small cavums Kautzky noted that 8 had fragmentary bits of lining cells. He concluded from his studies that the lining cells were morphologically so similar to ependymal cells that they might be related to them. From my own study of these cells I could not prove them to be of either ependymal or glial origin.

In freshly cut brains the cavum septi pellucidi contains a clear colorless fluid. Several attempts to collect this fluid for comparison with spinal fluid were unsuccessful. Some believe the fluid to be a transudate from the adjacent ventricles, others that it is just extracellular fluid. Van Wagenen [7] contended that there are lining cells of glial origin which actually secrete the fluid. So far it cannot be definitely said from a histologic standpoint that this is true. If the fragmentary lining cells are actually ependymal or glial cells, they could be the source of the fluid. The cells so far have been found in the small as well as in the hydropsic cavum and therefore can hardly be the sole cause of the pathologic enlargement of the cavum.

Pathologic changes are rarely associated with the cavum septi pellucidi. Van Wagenen and Aird [7] contributed an extensive report on the pathologic cavum and described three types: (1) a noncommunicating cavum, the walls of which are intact, (2) a communicating cavum, in which there are openings into the ventricular system and (3) a communicating cavum with dilation by later developing or superimposed hydrocephalus. Only the first type produced symptoms. Of the patients whose cases were reported by Van Wagenen and Aird only 5 had histories suggestive of symptoms which could have been associated with a pathologic condition

3. Smith, cited by Thompson.[2]

4. Pendergrass, E. P., and Hodes, P. J.: Ann. Surg. **101**:269, 1935.

5. Wolf, A., and Bamford, T. E.: Bull. Neurol. Inst. New York **4**:294, 1935.

6. Kautzky: Ztschr. f. Anat. u. Entwcklngsgesch. **108**:560, 1938.

7. Van Wagenen, W. P., and Aird, R. B.: Am. J. Cancer **20**:539, 1934.

of the cavum. They also reported 5 of Meyer's cases. Dandy [8] reported 2 cases of pathologic dilation of the cavum. An instance of hemorrhage into the cavum septi pellucidi was reported by Olsen.[9]

SUMMARY

The cavum septi pellucidi is a normal developmental structure produced by the lateral stretch of the corpus callosum.

The cavum is larger and is found more frequently in children than in adults (54 per cent of 50 unselected brains of infants and children, and 42 per cent of 50 unselected adult brains).

No anatomic communication with the ventricular system could be demonstrated in progressive sections of cavums from normal brains.

No definite ependymal or glial cells were found lining the cavums examined and no evidence of choroid plexus was seen in progressive sections of cavums.

Only on rare occasions is the structure responsible for clinical disturbances.

8. Dandy, W. E.: Arch. Neurol. & Psychiat. **25**: 44, 1931.

9. Olsen, C. W.: Bull. Los Angeles Neurol. Soc. **7**:152, 1942.

RENAL INJURY AND LYMPHATIC ATROPHY

LYMPHOPENIA AND ATROPHY OF LYMPHATIC TISSUE ASSOCIATED WITH ACUTE RENAL INSUFFICIENCY IN DOGS

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CHAPEL HILL, N. C.

During studies on the relation of diet and renal insufficiency to arterial disease in dogs [1] routine necropsies revealed diminution of the spleen, the thymus and the mesenteric lymph nodes. Histologic study of these organs as well as of the lymphatic tissue in the alimentary tract revealed the changes illustrated in figures 1 to 4. These changes consisted of a general "washing out" of lymphocytes and a curious change in the "germinal centers" that was different from the "normal resting phase," the "active phase" or "germinal center of Flemming," or the "regressive phase" or "reaction center of Hellman." The change most closely resembled the latter type of reaction, i.e., the "regressive phase." For lack of a better term it has been called "reticular arrest," for the cells that constitute the remnants of the "follicle" look like reticular cells. When viewed as a whole the process seems best summarized by the term "atrophy due to exhaustion." It is the purpose of this paper to show that this change is regularly associated with the production of acute renal insufficiency in adult dogs.

METHODS

Most of the dogs used for this report were originally used for studies on the relationship of diet and renal insufficiency to arterial disease. The general methods employed and much of the detailed data on these dogs have appeared in previous publications.[1] Seventy-eight normal adult dogs maintained on one or another of these diets—"standard," "kennel" or "lean meat"—were subjected to renal insufficiency in one of three ways—by subcutaneous injection of uranium nitrate (5.0 mg. per kilogram, 50 dogs), by intravenous injection of mercury bichloride (3.0 mg. per kilogram, 22 dogs) or bilateral nephrectomy (6 dogs). Eight of the dogs were made hypoproteinemic by plasmapheresis, 16 were made hyperproteinemic by injections of plasma, and 15 received intravenous injections of sodium citrate (the anticoagulant used in making the injections of plasma). The other 39 dogs were not subjected to any "plasma alteration"; i. e., the experimental variables in this group consisted only of diet and renal insufficiency.

Except in the 7 dogs protected against injury from uranium nitrate by injections of sodium citrate [2] and in the 3 dogs protected against injury from mercury bichloride by hypoproteinemia [3] death occurred in from three to seventeen days after renal insufficiency was produced, with terminal values for nonprotein nitrogen ranging from 178 to 710 and averaging above 300 mg. per hundred cubic centimeters. In each instance death was apparently due to "acute uremia." Promptly after the dogs died, necropsies were made, and sections taken from all of the organs and from many of the tissues were fixed in 4 per cent solution of formaldehyde and in Zenker's fluid. Paraffin sections of the material fixed in Zenker's solution cut at 7 microns were stained routinely with hematoxylin and eosin. Appropriate special stains were used in selected cases.

EXPERIMENTAL OBSERVATIONS

The gross and the histologic changes in the lymphatic tissues of these dogs subjected to acute renal insufficiency were not as striking as the term "atrophy due to exhaustion" implies. In the first few dogs in the series they went unnoticed, and it was only after necropsy of control dogs that we became impressed with the changes. These control dogs (20 dogs used for teaching purposes in pharmacology and physiology, 10 dogs that succumbed to accidents in the experimental group and 10 planned controls) frequently had big spleens with prominent malpighian bodies, but the spleens of the dogs dying of acute renal insufficiency were consistently small and contracted. In many of the controls the thymus was conspicuous, whereas in the dogs with renal injury only a small amount of indefinite "areolar thymic tissue" was found in the anterior superior mediastinum. And the lymph nodes, especially the mesenteric nodes, in the control group were larger and had thicker cortices than those in the experimental group.

From the Department of Pathology, University of North Carolina, Chapel Hill, and the Department of Laboratories, Watts Hospital, Durham, N. C.

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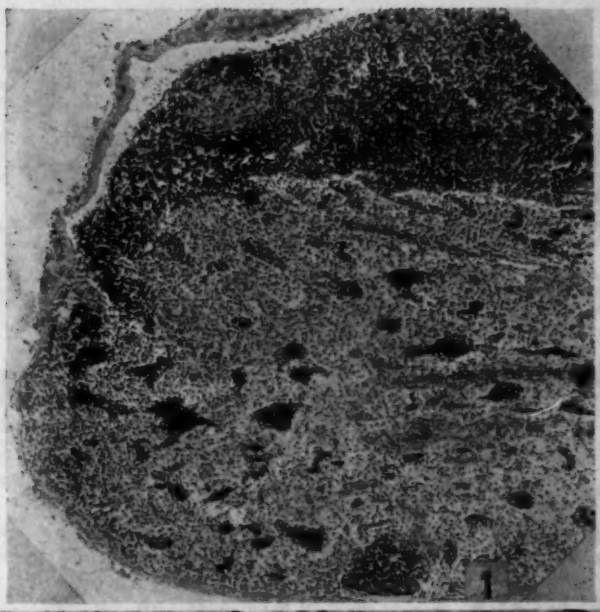
1. Holman, R. L.: Am. J. Path. **17**:359, 1941. Holman, R. L., and Hewitt, W. C.: Proc. Soc. Exper. Biol. & Med. **49**:58, 1942. Holman, R. L.: Am. J. Path. **19**:147 and 159, 1943; Necrotizing Arteritis in Dogs Related to Diet and Renal Insufficiency: V. Evidence for a Dietary Factor, ibid., to be published; Necrotizing Arteritis in Dogs Related to Diet and Renal Insufficiency: VI. Associated Lesions: Stomatitis, Gastro-Enteritis, and Pancreatic Fat Necrosis, ibid., to be published.

2. Donnelly, G. L., and Holman, R. L.: J. Pharmacol. & Exper. Therap. **75**:11, 1942.

3. Holman, R. L., and Donnelly, G. L.: J. Exper. Med. **76**:511, 1942.



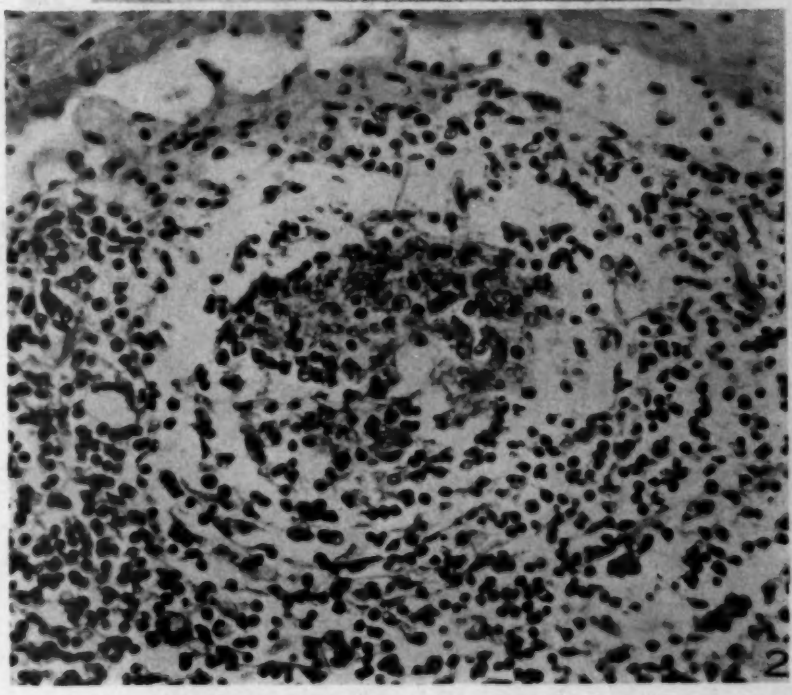
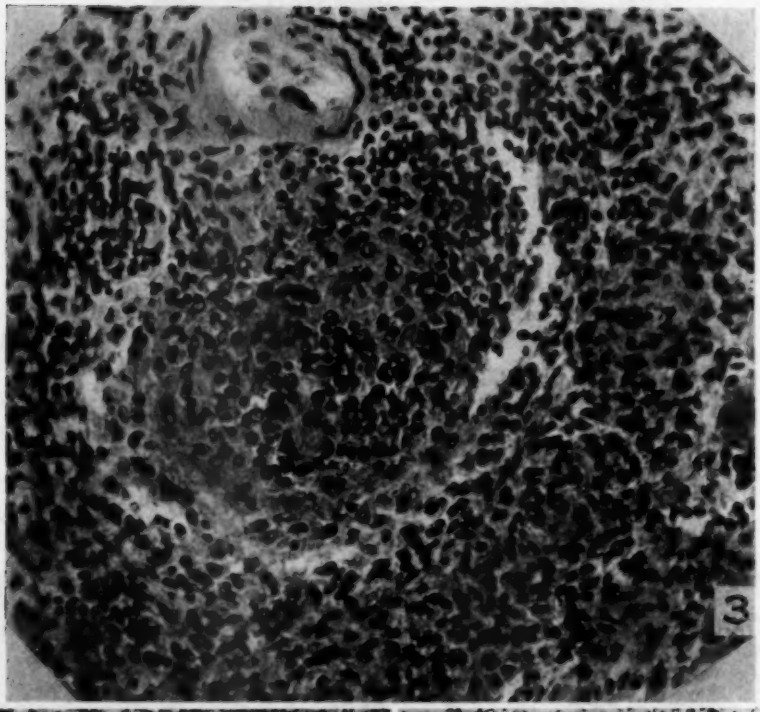
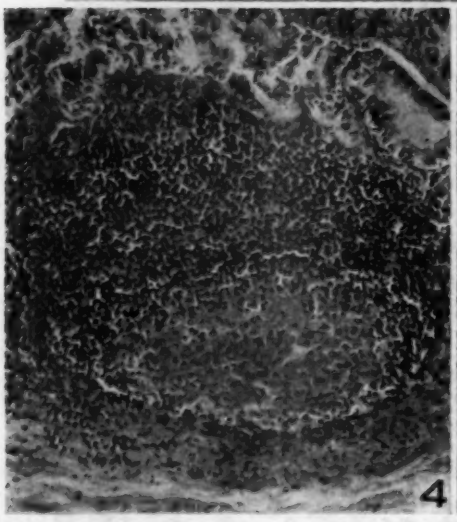


Fig. 1.—Mesenteric lymph node from an adult dog which died six days after receiving an injection of 5 mg. of uranium nitrate per kilogram. Note the general "washing out" of lymphocytes and the "fibrotic" appearance of the single erstwhile "follicle" in the remaining cortex. × 50.

Fig. 2.—Mesenteric lymph node from an adult dog which died eleven days after receiving an injection of 5 mg. of uranium nitrate per kilogram. Lymphocytes are sparse; some of the remaining nuclei show karyorrhexis, and reticular cells are coming into prominence. × 360.

Both sections were stained with hematoxylin and eosin.





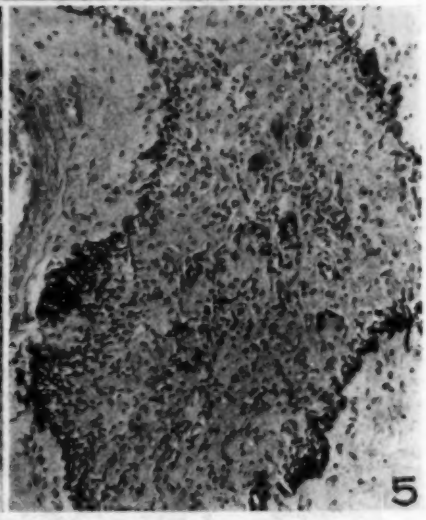


Fig. 3.—Spleen from an adult dog which died eleven days after receiving an injection of 5 mg. of uranium nitrate per kilogram. Lymphocytes have been largely replaced by reticular cells. × 325.

Fig. 4.—Peyer's patch from an adult dog which died sixteen days after receiving an injection of 5 mg. of uranium nitrate per kilogram. The changes are similar to those in figure 3. × 135.

Fig. 5.—Thymus of an 8 week old pup which died from inanition and ascarid infection. (No renal insufficiency was demonstrated in this dog.) Only a thin peripheral zone of small lymphocytes persists. × 140.

All sections were stained with hematoxylin and eosin.

It was the histologic changes in the "germinal centers" of the lymphatic tissues of the dogs subjected to acute renal insufficiency, however, that formed the most convincing evidence. These changes, illustrated in figures 1 to 4, are not easy to describe, but it has never been too easy to describe "normal" lymphatic tissues. The principal differences from "normal" can be listed as follows:

1. Decrease in number and size of "germinal centers."

2. Paucity of mature small lymphocytes in the cortex generally and especially about the "germinal centers."

3. Absence of the central zone of paler larger mononuclear cells (lymphoblasts?) in the "germinal centers" that remained.

4. Presence of large plump cells like epithelial cells about the central arterioles in the spleen and about the capillaries of the "germinal centers" in the lymph nodes. These cells are interpreted as reticular cells, and the silver stains are consistent with this interpretation. (Not to be confused with these changes are the "pseudotubercles"—presumably resulting from ascarid infection at an earlier age—occasionally found in the lymph nodes and other organs of adult dogs.)

I do not mean to leave the impression that these changes were uniform in every lymphatic tissue or in every follicle in any one of the lymphatic tissues, but the general trend was uniform. All in all it looked as if the lymphatic tissues had quit producing lymphocytes and that the lymphocytes remaining were on their way out. Once my attention was focused on these changes I was impressed with their almost invariable occurrence in the dogs dying of acute renal insufficiency.

Quantitative data supporting the gross and the histologic impression of atrophy of lymphatic tissue were obtained by weighing the spleens and the popliteal lymph nodes of 6 of the dogs that died of acute renal insufficiency. Table 1 shows that the average decrease in the weight of the spleens was 54 per cent and that the average decrease in the weight of the popliteal lymph nodes was 85 per cent (table 2). The control figure for weight of the spleen was obtained from dogs killed in physiologic experiments, and the control figure for weight of the popliteal lymph nodes was obtained from in vitro perfusion experiments to be reported at a later date. Even if one assumes that the control figure for weight of the popliteal nodes was 50 per cent too high (since these nodes could not be dissected entirely free from surrounding areolar tissue without injuring the cannulated trunk), the popliteal lymph nodes of the dogs dying with renal insufficiency weighed less than one third as much as did the controls.

TABLE 1.—*Weights of Spleens of Normal Dogs and Dogs Dying with Acute Renal Insufficiency*

Group	Number in Group	Average Weight of Spleens, Gm.*	Percentage of Normal Weight of Spleen
Normal dogs.....	11	26.0 (18.0-42.5)	100
Dogs with acute renal insufficiency.....	6	12.1 (7.1-20.3)	46

* All weights were corrected for a total body weight of 10.0 kilograms.

TABLE 2.—*Weights of Popliteal Lymph Nodes of Normal Dogs and Dogs Dying with Acute Renal Insufficiency*

Group	Number in Group	Average Weight of Popliteal Lymph Nodes, Gm.*	Percentage of Normal Weight of Nodes
Normal dogs.....	15	1.04 (0.7-2.0)	100
Dogs with acute renal insufficiency.....	6	0.16 (0.1-0.2)	15

* All weights were corrected for a total body weight of 10.0 kilograms.

The next point of interest was the relative and particularly the absolute number of lymphocytes in the blood, for it is generally agreed that most of the lymphocytes in the blood come from these lymphatic tissues. It has repeatedly been shown that over two times the total number of blood lymphocytes are poured into the circulation via the thoracic duct alone each day.[4] If the depots from which these cells come are exhausted and if the pathways of removal are unaffected, the number of circulating lymphocytes should decrease. That there was a significant drop in each of the 10 dogs studied is evident from figure 6. In the first few days after renal injury the total leukocyte and lymphocyte counts fluctuated considerably but the final lymphocyte count of every dog except 1 was below the normal range, and this dog did not die until five days later. The average decrease in number of blood lymphocytes amounted to 68 per cent, and in 2 of the dogs for which agonal counts were obtained only about 10 per cent of the control number of lymphocytes remained in circulation immediately before death. As to the 2 dogs with high lymphocyte counts at the time of renal injury, both had alteration of their plasma proteins (hyperproteinemia in one and hypoproteinemia in the other). The "average" dog—indicated by the heavy line in figure 6—died on the twelfth day after renal injury and showed a fall in total lymphocytes from 3,400 to 1,100 per cubic millimeter.

In summary, the weights of the spleens and popliteal lymph nodes and the blood counts support the gross and histologic impression of "exhaustion atrophy" of lymphatic tissue. When all

4. Rous, P.: J. Exper. Med. **10**:537, 1908. Davis, B. F., and Carlson, A. J.: Am. J. Physiol. **25**:173, 1909-1910. Bunting, C. H., and Hutson, J.: J. Exper. Med. **33**:593, 1921. Yoffey, J. M.: J. Anat. **70**:507, 1936.

the data on the 78 dogs were analyzed, six points of interest emerged:

1. The method by which renal insufficiency was produced—subcutaneous injection of uranium nitrate, intravenous injection of mercury bichloride or bilateral nephrectomy—did not influence the incidence of these atrophic changes in the lymphatic tissues.

2. The atrophic changes were as marked in dogs subjected to bilateral nephrectomy as in dogs given uranium or mercury; hence heavy metal was not the responsible agent.

3. The diet on which the dogs were maintained prior to and after renal injury—"standard," "kennel" or "lean meat"—did not affect the atrophic changes in the lymphatic tissues.

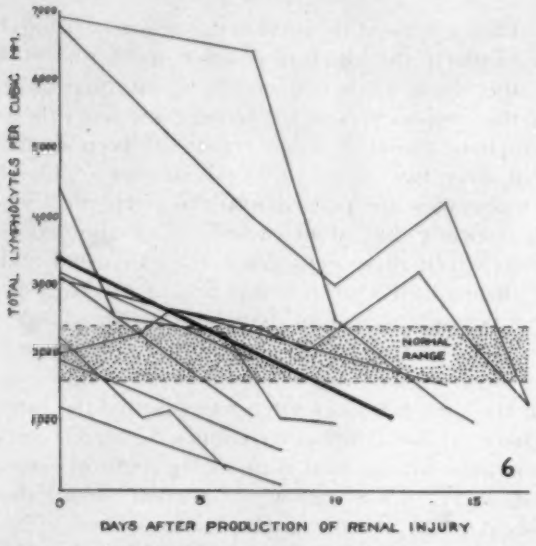


Fig. 6.—Blood lymphocyte counts following the production of acute renal insufficiency in adult dogs.

4. Other procedures — plasmapheresis, injections of plasma or injections of sodium citrate—were without effect on the lymphatic tissues except so far as they protected against renal insufficiency; e.g., hypoproteinemia protects dogs against a lethal dose of mercury bichloride,[3] and in 3 of these dogs, killed eight, fourteen and forty-five days after the injection of the heavy metal compound no atrophy of lymphatic tissues was found.

5. That the changes are reversible is shown by the following type of experiment: Dogs receiving intravenous injections of sodium citrate survive a minimum lethal dose of uranium nitrate,[2] but during the first week after the injection of the heavy metal compound the urinary, blood chemical and anatomic findings all indicate renal damage. Such dogs killed during the first week after the injection of the heavy metal compound showed atrophy of lymphatic tissues while those killed six to thirty-six months later, i.e., after recovery from renal injury, showed "normal" lymphatic tissues.

6. The anatomic changes depicted in figures 1 to 4 have been observed as early as four days after bilateral nephrectomy. These observations confirm recently published reports in indicating a potential rapid turnover in lymphatic tissues.[5]

COMMENT

The experimental data indicate that acute renal insufficiency in adult dogs is associated with atrophy of lymphatic tissues and lymphopenia. But are the atrophic changes related directly to renal damage or are they due to secondary effects of renal insufficiency, such as inanition, acidosis and infection? The data available from the present investigation are against the view that these secondary effects cause the condition in question.

Following the production of renal insufficiency, the dogs stopped eating two to ten days later or two to eight days before they died. The effect of inanition on lymphatic tissues therefore has to be considered in some detail. A review of the literature on this point revealed variable results,[6] none of which were suitable to serve as proper controls on the present studies, and the following experiments were performed. Control blood counts were obtained on 5 adult dogs (ranging in age from 2 to 6 years and in body weight from 6 to 12 kilograms) which had been in the kennel for at least one month and were apparently free from any infection. All food was withheld but free access to water was continued. The blood counts were repeated after seven to eighteen days of fasting and at later intervals in 3 of the dogs. Each count was made on blood dripping freely from a 20 gage needle inserted in one of the external jugular veins. Two of the dogs were returned to kennel diet after twelve and eighteen days of fasting, and the other 3 were made to fast until they died (on the forty-fi :, sixtieth and eighty-fourth day).

The control and "fasting" blood counts are given in table 3. While the total white blood cells and especially the total lymphocytes per cubic millimeter in 2 of the dogs made to fast until death did show a definite drop, none of the counts taken from the seventh to the eighteenth day of fasting—the time corresponding to that of death from renal insufficiency in the experiments reported in the foregoing pages—was significantly outside of the normal range. Losses of body

5. Conway, E. A.: Anat. Rec. **69**:487, 1937.

6. Jackson, C. M.: Effects of Inanition and Malnutrition upon Growth and Structure, Philadelphia, P. Blakiston's Son & Co., 1925.

weight in this control fasting group during this period averaged 26 per cent while in the 10 dogs whose counts are plotted in figure 6 the losses in body weight from the time renal insufficiency was produced until death averaged 13 per cent and in no instance amounted to as much as 20 per cent. Anatomic studies disclosed "normal" lymphatic tissues in the 2 dogs refed to original weight and atrophic changes in the lymphatic tissues of the 3 dogs fasting until death. The atrophic changes, however, were more diffuse and corresponded to the general decrease in body weight. Specifically, no areas of "reticular arrest" such as those illustrated in figures 1 to 4 were found in the lymphatic tissues of these 3 dogs. The only histologic changes encountered that are comparable

TABLE 3.—*Effect of Fasting on Body Weights, Total White Blood Cell Counts and Total Blood Lymphocyte Counts of Adult Dogs*

Dog	Period of Fast, Days	Body Weight, Kg.	Total White Blood Cells per Cu. Mm.	Per-centage Lympho-cytes	Total Lympho-cytes per Cu. Mm.
43-40	0	11.8	11,800	42	4,956
	7	10.5	10,200	40	4,080
	12	9.7	16,800	26	4,368
	—32*	11.5	14,300	36	5,148
43-45	0	6.0	14,700	20	2,940
	14	4.3	8,700	26	2,262
	—32*	6.2	8,600	24	2,064
43-46	0	8.6	20,400	17	3,468
	18	6.4	8,400	25	2,100
	40	4.4	5,700	22	1,254
43-47	0	14.1	6,800	27	1,836
	18	11.3	16,950	34	5,763
	45	9.0	9,200	40	3,680
	81	6.4	10,200	21	2,142
43-49	0	11.6	12,600	15	1,890
	18	8.2	7,500	23	1,725
	45	5.9	8,700	21	1,827
	59	5.2	9,900	12	1,188

* The dog was refed until a normal weight was attained.

to the atrophic changes pictured in figures 1 to 4 have been in pups 1 to 2 months of age with ascarid infection and inanition but with no demonstrated renal insufficiency. Figure 5 illustrates these changes in the thymus of 1 pup. These changes in pups (accidental involution of Hammar?) may be related to differential demands for growth and not to inanition in general as this term is applied to adult dogs. At any rate I feel confident that the atrophic changes in the lymphatic tissues of adult dogs dying from acute renal insufficiency occur much sooner and are more marked than can be accounted for on the basis of inanition.

No blood counts or weights of lymphatic tissues that bear on the question of acidosis are available, but the indirect evidence is against this factor. Intravenous injections of sodium citrate given before and after the production of renal insufficiency do not prevent the acidosis associated with renal insufficiency but do lessen it to some extent.[2] The atrophic changes in the lymphatic tissues of the dogs receiving injections of sodium citrate were just as marked as in those not receiving such injections. I have not found in the literature any reports in which acidosis is shown to be associated with lymphopenia. I have not studied this phase of the problem directly.

Acute inflammatory processes have been frequent in these dogs; in fact, they were the subject under investigation when the changes in the lymphatic tissues became apparent. These inflammations—acute necrotizing arteritis, acute necrotizing stomatitis, acute hemorrhagic gastroenteritis and acute necrosis of pancreatic fat—have all been described in previous publications.[1] The majority of dogs in this series have shown one or more of these inflammatory processes. In none of them, however, has such a process affected lymphatic tissue directly, and the data on some of the dogs without these inflammatory changes, e.g., those protected against a lethal dose of uranium nitrate by repeated intravenous injections of sodium citrate,[2] have shown clearly that the lymphopenia and the atrophy of lymphatic tissues can occur independently of all these acute inflammatory changes. Further it seems improbable that infections could have played an important role in the changes in the lymphatic tissues, for the experiments were too acute, and the infections that lead to recognized changes in lymphatic tissues are all chronic and do not lead to atrophy and lymphopenia but to hyperplasia and lymphocytosis.

So much for the negative side: I feel confident that these changes in the lymphatic tissues are not due to inanition or infection and are probably not related to acidosis. The only known factor common to all of the dogs that have shown these atrophic changes is acute renal insufficiency. This throws the mechanism into the field of "disturbed metabolism," but this vague expression means little or nothing unless one can track down the faulty mechanism. Is the pathogenic ("alymphocytogenic" and lymphopenic) factor a deficiency of a necessary (maturation?) substance that is normally formed in the kidney or is it the presence of a toxic substance derived from the damaged kidney? The fact that the changes are just as marked in dogs subjected to bilateral nephrectomy would favor the former view. Further the appearance of the "germinal centers" in "reticular arrest" plus the reversibility of the changes with regeneration of the kidney (in dogs protected against uranium nitrate by injections of sodium citrate) are both compatible with defi-

ciency of a maturation factor. This line of reasoning suggests hormonal activity by the kidney; I believe the evidence is sufficient to warrant search for a lymphocytic maturation factor in the renal cortex. I realize, however, that many other explanations are possible.

One bit of indirect evidence bearing both on the hypothetic hormonal substance and on the general problem of the function of the mammalian lymphocyte might be considered at this point. If renal insufficiency leads to "exhaustion atrophy" of lymphatic tissues with resulting lymphopenia, it is possible to explain the anemia of nephritis on the view of Maximow,[7] Jordan,[8] Yoffey and Drinker,[9] Kindred [10] and others that some of the blood lymphocytes are filtered out in the bone marrow for conversion into erythrocytes. The other and possibly more widely accepted view regarding the anemia of nephritis is that a "toxic factor" depresses erythropoiesis. Attempts to date to isolate this "toxic factor" from the blood or the kidneys of patients with nephritis have not yielded decisive results. Possibly none of these experiments has taken into consideration the "factor of safety" afforded by normal lymphatic tissues (i.e., if the view of Maximow and others that the lymphocyte plays a part of the normal cycle of erythropoiesis is correct). It is not advisable at this time to get involved in estimations of the total amount of lymphatic tissue in the body, the number of lymphocytes delivered to the circulation each day and the part that these cells could play in maintaining the red blood cell level under normal and various pathologic conditions, but it can be stated that this "factor of safety" (if such it be) is far from negligible. The meager data that are available—terminal hematocrit values—do not support this hypothesis, but it may well be that a period longer than two weeks is required to deplete this "reservoir." Possibly the same factor depresses both erythropoiesis and lymphopoiesis.

7. Maximow, A.: Arch. f. mikr. Anat. **76**:1, 1910.
8. Jordan, H. E.: Anat. Rec. **73**:227, 1939.
9. Yoffey, J. M., and Drinker, C. K.: Anat. Rec. **74**:417, 1939.
10. Kindred, J. E.: Am. J. Anat. **71**:207, 1942.

It is not known whether these atrophic changes in the lymphatic tissues of adult dogs can be produced by other mechanisms, whether acute renal insufficiency leads to atrophy of lymphatic tissues and lymphopenia in other species of animal or whether chronic renal insufficiency of varying degree is associated with these same changes. I am now in the process of analyzing human clinical and necropsy data.

SUMMARY

Gross and histologic changes interpreted as "exhaustion atrophy" were consistent findings in the lymph nodes, the spleen, the thymus, Peyer's patches and solitary follicles in a group of 78 adult dogs which were subjected to acute renal insufficiency (50 by subcutaneous injection of uranium nitrate, 22 by intravenous injection of mercury bichloride and 6 by bilateral nephrectomy).

Gross weights of the spleen and popliteal lymph nodes in a limited number of these dogs confirmed the diminution in size of these lymphatic tissues.

Lymphocyte counts on the circulating blood also showed a sharp reduction. In 2 of the dogs on which agonal counts were made the lymphopenia amounted to about 10 per cent of the level noted previous to renal injury. The average reduction for the group was to 32 per cent of the control level.

Are the atrophic changes related directly or indirectly to renal damage? A part of the evidence presented militates against the view that they may be related to some of the states that follow renal insufficiency, such as inanition, acidosis and infection. If the disturbance is not secondary to one of these known indirect factors, is the pathogenic factor a deficiency of a necessary (maturation?) substance or the presence of a toxic substance? A part of the evidence presented favors the former view.

CONCLUSION

Acute renal insufficiency in adult dogs is associated with lymphopenia and atrophy of lymphatic tissue.

GENETIC ANALYSIS OF THE INDUCTION OF TUMORS BY METHYLCHOLANTHRENE

VII. PRIMARY CARCINOMA OF THE LIVER FOLLOWING SUBCUTANEOUS INJECTION OF METHYLCHOLANTHRENE IN MICE

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In unselected NHO mice a subcutaneous injection of methylcholanthrene dissolved in sesame oil usually results in the local appearance of (*a*) fibrosarcoma, (*b*) rhabdomyosarcoma or (*c*) epidermoid carcinoma, presumably depending in part on the type of cells with which the carcinogen comes into contact.[1] Selection toward resistance to this local induction of tumors, following an original outcross, resulted in three phenomena as follows: (*a*) The latent period for the appearance of the local tumors enumerated had been considerably extended (from one hundred and twenty-six days in the F_4 to two hundred and fifteen and two-tenths days in the F_{12} generation), or (*b*) the mice now lived a normal life span without the occurrence of any neoplasm, or (*c*) tumors now developed at sites distant from the site of injection (a subcutaneous site on the right side of the body), and these tumors were unlike the tumors induced in mice in the early generations. Among these remotely occurring neoplasms were 19 diagnosed as primary carcinoma of the liver. Mice showing cancers of other organs metastatic to the liver are not included in this report.

MATERIALS AND METHODS

All mice used in this experiment were of the NHO descent and belonged to the same series as those described in previous reports.[1] The NHO mice were originally produced by tandem crosses between mice of the CBA, the JK and the N inbred strains. The idea behind the production of the NHO strain was to obtain a group of mice which would show the greatest possible degree of biologic variability without the interfering incidence of spontaneous tumors. Up to the present time spontaneous tumors have been infrequent, as reported previously.[1f] The incidence of all types of spontaneous tumors with the exception of those of the lung is less than 1 per cent. Bronchiogenic carcinoma is the only spontaneous tumor to which the NH (or the NHO) stock shows any significant susceptibility (starting at 325 days of life and increasing in numbers up to 30 per cent of all mice by 575 days of life). In a very small number of these mice mammary tumors and leukemia have been observed.

At 60 days (plus or minus 1 day) all mice were given on the right side of the body a subcutaneous injection of 1 mg. of methylcholanthrene dissolved in 0.1 cc. of sesame oil. Mice were kept either as breeders or as nonbreeders on a diet of Nurishmix supplemented with a mixture of wheat and oats, enriched Bond bread soaked in milk and at intervals washed lettuce.[2] Weekly observations were made on all animals, and mice were killed either as soon as it was apparent that the local tumor was definitely growing or as soon as any disorder was shown, such as emaciation, ruffled hair and respiratory wheezing. On every mouse that died an autopsy was made within a few hours after death.

The present survey of tumors occurring in NH or NHO mice following the subcutaneous injection of methylcholanthrene is based on observation of 4,000 mice. The types of tumors observed included (1) fibrosarcoma, (2) rhabdomyosarcoma, (3) epidermoid carcinoma, (4) adenocarcinoma of the mammary gland, (5) adenocarcinoma of the pyloric end of the stomach and (6) adenocarcinoma of the lungs. To this list may now be added carcinoma of the liver (malignant hepatoma), 19 cases of which are reported in this paper. The extensive experiment on 4,000 mice (between the F_4 and the F_{12} generation) may be divided into two parts. The first part consisted of observations on 2,000 unselected mice (the NH) treated with methylcholanthrene. By this is meant that every mouse of

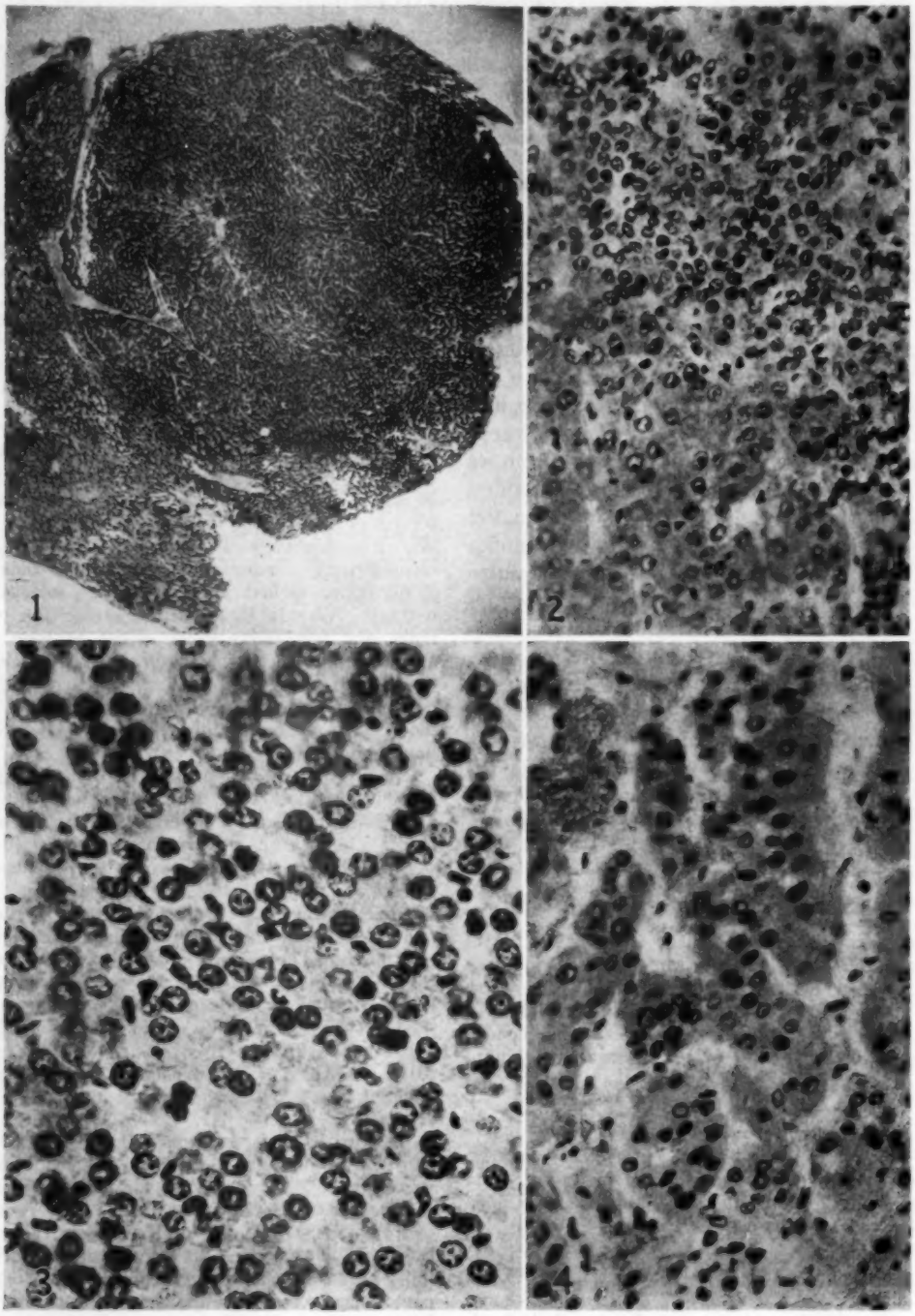
From the Department of Anatomy, Yale University School of Medicine.

This experiment was made possible by grants from the Anna Fuller Fund and the Jane Coffin Childs Memorial Fund for Medical Research.

1. (*a*) Strong, L. C.: Am. J. Cancer **39**:347, 1940; (*b*) Cancer Research **1**:572, 1941. (*c*) Strong, L. C., and Williams, W. L.: ibid. **1**:886, 1941. (*d*) Strong, L. C.; Collins, V. J., and Durand, E. A.: ibid. **3**:21, 1943. (*e*) Strong, L. C.: Arch. Path. **36**:58, 1943. (*f*) Williams, W. L., and Strong, L. C.: A Genetic Analysis of the Induction of Tumors by Methylcholanthrene: VI. Epidermoid Carcinomas and Associated Tumors in Mice of the F_4–F_7 Generations of the NH Descent, Cancer Research, to be published.

2. Nurishmix is a product of the Pratt Food Company. Its guaranteed analysis is: crude protein not less than 20 per cent, crude fat not less than 6.50 per cent, crude fiber not more than 3.50 per cent, carbohydrates 51.50 per cent and nitrogen-free extract not less than 48 per cent. Its ingredients are dried buttermilk, beef scrap, wheat germ meal, rolled oats, calcium pantothenate, molasses, iodized salt 1 per cent and cod liver oil U. S. P. Bond Bread is the product of the General Baking Company. Its guaranteed ingredients are fine wheat flours with added vegetable proteins, granulated sugar, skim milk solids, table salt, pure shortening, yeast and yeast nutrients, malt syrup and pure water. It is enriched with vitamin B_1 vitamin B_2 (riboflavin) and nicotinic acid.

the early hybrid generations (F_4 to F_8) received an injection of the carcinogen. The second part consisted of observations on mice in which selection toward resistance to the induction of tumor at the site of injection was being carried on (the NHO mice, numbering 2,000). In the unselected NH mice no hepatic tumors were found. These data are now complete. In the selected NHO mice 19 tumors of the liver, diagnosed as primary carcinoma of the liver, have so far been found. Six hundred and thirty-three of the 2,000 mice



1, photomicrograph of a nodular type of hepatic tumor occurring in mouse 2Q28, male, at the one hundred and sixty-eighth day following a subcutaneous injection of methylcholanthrene; × 29. *2*, photomicrograph of the invasive type of hepatic tumor occurring in male mouse 166395 at six hundred and thirty-four days; × 312. *3*, photomicrograph of carcinomatous hepatoma from mouse 2Q28, male; × 208. Note the large number of mitoses (same liver as shown in fig. 1). *4*, photomicrograph of hepatoma from mouse 166638, male, at five hundred and ninety-eight days; × 396. Note the giant cell mass near the center.

in the selection experiment are still alive and free of any obvious tumors at ages from 160 to 817 days. The opinion is held that the incidence of hepatic tumors here reported (19 in 1,367 mice) is no true final index. This opinion is based on the thesis developed partly in this paper, especially with regard to the occurrence of adenocarcinoma of the stomach, that the surviving population of 633 mice is a selective group (by the suppression of local tumors) and the mice, especially toward the end of the series, are having a greater and greater opportunity of developing other tumors remote from the site of injection, some of which may be cancerous tumors of the liver.

The objects of the present communication are (1) to place on record that carcinoma of the liver (malignant hepatoma) may be produced experimentally by the subcutaneous injection of methylcholanthrene and (2) to develop a method based on a genetic concept by which this particular neoplasm may be produced in ever increasing numbers.

RESULTS

These 19 primary hepatic cancers have occurred in 17 mice (9 females and 8 males) at an average age of 406.2 days. One mouse had three separate primary tumor masses in the liver. The range of time for the appearance of hepatic carcinoma was from one hundred and sixty-eight to five hundred and sixty-four days after the injection of methylcholanthrene. No hepatic tumors have so far appeared in 500 control NHO mice, although the data on mice beyond 625 days of life are not at present complete. The average age of 406.2 days is considerably earlier than the 602.3 days, which is the average age of incidence of spontaneous hepatoma occurring in C_3H and CBA mice.

The tumor masses were either nodular (fig. 1) or had irregular margins showing invasion into normal-appearing liver tissue (fig. 2). In the nodular type there were irregular hyperchromatic areas interspersed with areas that appeared to retain the staining capacity of normal liver. The structure of normal liver was greatly distorted near the tumor areas, the invading mass being made up of irregularly shaped tumor cells (fig. 4). Multinucleated giant cells were common. Mitotic figures were numerous, especially in the hyperchromatic areas. In one low power field of a hyperchromatic area twenty mitotic figures were counted (fig. 3). The tumors were somewhat similar to those spontaneously occurring in CBA and C_3H mice but appeared to be more malignant as judged by (*a*) the presence of invasion into normal liver and (*b*) the greater frequency of mitoses in the tumors of the present methylcholanthrene-treated series of animals. In fact, the tumors appear to be as malignant as those in rats which had been induced by the administration of butter yellow or of one of the chemicals related to it (Yoshida [2a]).

COMMENT

Three classes of chemicals have led to some degree of success in the production of hepatic tumors in experimental animals as follows: (*a*) butter yellow, orthoaminoazotoluene and the closely related chemicals acetylorthoaminoazotoluene, and paradimethylaminoazobenzene; (*b*) the carcinogens derived from phenanthrene, such as methylcholanthrene and 3,4-benzpyrene, and (*c*) the radioactive substances, such as radium bromide. A few references to the extensive literature on each of these three classes may be cited.

(*a*) Yoshida [2a] reported the development of hepatoma in all white rats that survived the two hundred and sixty-sixth day following the introduction of orthoaminoazotoluene into the food in the concentration of 0.1 per cent (4 rats of the original 26 started on the experiment). This observation has been extended to guinea pigs and to mice but not apparently to rabbits. Andervont [3] reported hepatic tumors in mice of the I, C_3H, C and A strains which had received subcutaneous injections of 10 mg. of orthoaminoazotoluene. He stated, however, that "the results suggest that in these strains susceptibility to liver growth induced by 2-amino-5-azotoluene is not correlated with susceptibility to spontaneous hepatomas." Seligman and Shear [4] obtained tumors of the liver as well as of the mammary glands by the subcutaneous injection of 10 mg. of acetylorthoaminoazotoluene but expressed the opinion that they were probably of a spontaneous nature (C_3H mice used, which show a high incidence of spontaneous hepatoma as well as of adenocarcinoma of the mammary gland).

(*b*) Lorenz and Andervont [5] reported tumors of the liver in C_3H mice following the subcutaneous injection of 0.8 mg. of 1,2,5,6-dibenzanthracene dissolved in lard. Andervont [6] reported hepatic tumors in C_3H mice following the subcutaneous injection of 0.0625 to 0.25 mg.

2a. Yoshida, T.: Proc. Imp. Acad. Japan **8**:464, 1932.

3. Andervont, H. B.: Pub. Health Rep. **54**:1529 and 1986, 1939.

4. Seligman, A. M., and Shear, M. J.: Unpublished data; cited by Hartwell, J. L.: Survey of Compounds Which Have Been Tested for Carcinogenic Activity, Federal Security Agency, United States Public Health Service, 1941.

5. Andervont, H. B., and Lorenz, E.: Pub. Health Rep. **52**:637 and 1931, 1937.

6. Andervont, H. B.: Pub. Health Rep. **54**:1158, 1939.

of 20-methylcholanthrene in lard. He maintained that "definite conclusions are not justified as to whether hepatomas are induced by dibenzanthracene or methylcholanthrene." Strong, Smith and Gardner [7] reported hepatoma in CBA mice following the subcutaneous injection of 0.1 per cent 3,4,5,6-dibenzocarbazole in sesame oil (0.2 cc. for six injections). Similar treatment of strain A mice gave no hepatic tumors. Boyland and Huntsman Mawson [8] reported cholangiomatous growths in livers of heterogeneous mice with the same chemical injected intraperitoneally.

(*c*) Daels and Biltris [9] reported 2 tumors diagnosed as epithelioma of the biliary tract following the local injection into guinea pigs of 0.2 micromilligram of radium sulfate per square millimeter on collodion strips. Petroff and Krotkina [10] reported 2 tumors diagnosed as adenocarcinoma of the liver (12 guinea pigs in experiment) following the installation of 0.4 to 1.8 micromilligrams of radium in a glass tube into the gallbladder.

The survey of the literature and the analysis of the present data lead to the conclusion that from the point of view of genetics hepatic tumors can be produced experimentally in at least two ways: (1) by administering one of several chemicals to mice which normally give rise to spontaneous hepatic tumors, such as mice of the C_3H and CBA strains—in this case the injected material shortens the latent period for the appearance of the spontaneous tumors, thus increasing somewhat the incidence of hepatic tumors—or (2) by administering one of several chemicals to experimental animals that do not normally give rise to spontaneous hepatic tumors. In the first case it may be stated that the inducing material *augments* or *supplements* genetic susceptibility; in the second, that the inducing chemical *replaces* genetic determination. This seemingly contradictory phenomenon may be explained in part on the basis that susceptibility and resistance to cancer are determined by quantitative and not qualitative differences. As far as I know there are no two strains of experimental mice that differ absolutely in susceptibility and resistance to cancer (the difference between + and — or 100 and 0 per cent). Susceptibility and resistance to cancer are relative terms only. This concept of susceptibility certainly applies to adenocarcinoma of the mammary gland [11] and may be applicable to other types of neoplasms, including hepatoma (both cancerous and noncancerous).

A practical application of the concept of quantitative susceptibility and resistance to tumors has already been made in the investigation of adenocarcinoma of the pyloric end of the stomach of the mouse. That is, this type of neoplasm has never been found spontaneously in mice of the unselected NH strain (same strain and the lineal NHO descent as used in this investigation). In NHO mice after partial selection toward resistance to local induction of tumors has been accomplished, the subcutaneous injection of methylcholanthrene dissolved in sesame oil (1 mg. in 0.1 cc. of oil) has so far produced 65 pyloric neoplasms diagnosed as adenocarcinoma. By keeping the descendants of these animals showing induced pyloric adenocarcinoma a selected subline has been developed in which this specific neoplasm is beginning to occur not only in the presence of methylcholanthrene but also spontaneously (6 cases already observed). Thus there seems to be some degree of relationship between the appearance of this specifically induced and the spontaneous histologically similar neoplasm when without further investigation there appeared to be none. In this instance the carcinogen has been used as a tool for the detection of a genetic determiner or determiners that could not otherwise be detected (unpublished data). One interpretation of these facts is as follows: In the unselected NHO mice there is a tendency to give rise to spontaneous and to induced carcinoma of the pyloric region of the stomach. Only in certain individuals is this tendency strong enough to respond with gastric neoplasia in the presence of methylcholanthrene. During the process of selection over several generations (made possible only in hybrid mice such as the NHO) this intrinsic tendency becomes so enhanced that now the mice not only give rise to increasing numbers of methylcholanthrene-induced pyloric cancers but also to similar cancers spontaneously.

The conclusion is valid, as previously expressed,[16] that in the last analysis the types of tumors obtained either spontaneously in genetically controlled animals or after the administration of any one of the different carcinogens are due not only to the structural configuration of the molecule of the inducing chemical (for the induced tumors) or of a hypothetic carcinogen for the spontaneous tumors but also to the constitution of the mouse or

7. Strong, L. C.; Smith, G. M., and Gardner, W. U.: Yale J. Biol. & Med. **10**:335, 1938.

8. Boyland, E., and Huntsman Mawson, E.: Biochem. J. **32**:1460, 1938.

9. Daels, F., and Biltris, R.: Bull. Assoc. franç. p. l'étude du cancer **20**:32, 1931.

10. Petroff, N., and Krotkina, N.: Ztschr. f. Krebsforsch. **38**:249, 1933.

11. Strong, L. C.: Brit. J. Exper. Path. **17**:60, 1936.

experimental animal, controlled to a great degree by specific genes. "The directional path taken by the individual in the induction of specific tumor types is the resultant of the genetic constitution of the individual and is determined by heredity." [18a] In view of the findings with gastric carcinoma, this concept may also be applicable to primary carcinoma of the liver. Selection of the descendants of some of the mice in which primary malignant hepatoma developed is being continued in the laboratory, and the incidence of tumors (especially of the liver), the spontaneous as well as the methylcholanthrene-induced type, will be reported when available.

SUMMARY

Selection toward resistance to local induction of tumors by methylcholanthrene has led to the appearance of 19 cases of cancerous hepatoma (primary carcinoma of the liver) in 17 mice of the NHO descent. Hybrid mice, such as those of the NHO descent, are endowed with the capacity to give rise to a great number of tumors of various parts of the body following the subcutaneous injection of methylcholanthrene. These tendencies could be detected only when the development of tumors of such types as fibrosarcoma, rhabdomyosarcoma and epidermoid carcinoma at the sites of injection was eliminated or suppressed by genetic selection toward resistance to induction of such local tumors. There appears to be a relation of the incidence of spontaneous to that of induced tumors and of genetics to both.

Dr. H. S. Zimmerman verified the pathologic diagnoses of the tumors of the liver discussed in this paper.

Case Reports

SACROCOCCYGEAL CHORDOMA WITH METASTASES

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In 1846 Virchow [1] observed a tumor in the spheno-occipital region at the base of the skull. It was composed of groups of large vesicular cells with chromatin-rich nuclei, situated within the meshwork of a delicate fibrous stroma. Occasionally these foamy cells, which Virchow called physaliferous cells, formed rosette-like symmetric structures. In 1858 Müller [2] described rests of the chorda dorsalis in man from which tumors of this type may have originated and called the tumors "chordoma." This view of their origin was confirmed by Ribbert,[3] who had been able to produce tumors resembling chordoma by injuring the intervertebral disks in rabbits so that particles of nucleus pulposus proliferated.

Feldmann [4a] and Mazzia [4b] were the first to describe a similar growth in the sacral region and refer to it as chordoma after systematically excluding the diagnosis of myxosarcoma or colloid carcinoma. The alveolar structure and the mucin present in only minimal quantities within the intracellular vacuoles though found in great abundance within the interalveolar and intercellular substance were the chief points used to rule out the diagnosis of myxosarcoma. The large size of the vacuoles and their emptiness, the sparsity of mitoses and the presence of capillaries within alveolar cell groups made the diagnosis of colloid carcinoma unlikely.

Fischer,[5] the second to report sacral chordoma, originated the idea that in accepting the connection between tumors of this type and chorda rests, chordoma could be expected to arise at any point throughout the entire extent of the chorda dorsalis.

Although opinions as to the embryonal origin of the anlage of the chorda dorsalis still differ, it is for the most part held that the primitive notochord originates from the entoderm. Physiologic rests in the form of nucleus pulposus and similar cell nests within the ligamentum apicis dentis have been traced repeatedly, and the relationship of chordoma to these rests has been definitely established. Many tumors diagnosed as chordoma have been reported as incidental findings since they often do not attain any considerable size and may cause no symptoms. These noncancerous forms of chordoma are usually situated at the clivus Blumenbachii; this is the site of predilection also for the cancerous forms, which are being more and more frequently reported. Their origin at any other site, such as the nasopharynx or the cervical, the thoracic or the lumbar region of the spinal column, is rare.

The sacrococcygeal region, however, is the next most frequent site for chordoma. Since Feldmann's report of a case of cancerous chordoma in this region in 1910, 82 such cases could be traced in the world literature up to date. Forty-four cases were collected by Podlaha and Pavlica [6] to November 1927. I was able to find reports of another 38 cases from that time to the present. The tumors were all locally invasive and almost invariably recurred after surgical removal, and did not react well to roentgen treatment. Some of the tumors attained a considerable size (up to that of a man's head). Metastases to distant organs were found in only 10 cases; brief summaries of the reports of these cases follow.

1. Curtis and Lefort [7]: Several cases were reported in which tumors of the sacrococcygeal region had developed. The authors ascribed the origin of these tumors to the perithelium of Luschka's glands. The histologic description, however, resembled closely the one pathologists now apply to the tumors arising from the notochord. In a case cited from the English literature [8] the tumor was called teratoma. It had slowly developed in a 57 year old man over a period of thirty-five years. Metastases had formed in the right groin in the form of two nodules the size of duck eggs. These had been present for twenty-three and twenty-five years respectively. The French authors considered the history and the postmortem observations similar to those in the cases they had reported as instances of tumor arising from Luschka's glands. It now seems that these neoplasms had the characteristics of sacrococcygeal chordoma, one of them showing the unusual formation of metastases.

2. Pototschnig [9]: A 40 year old blacksmith complained of a slowly growing tumor for ten years. Roentgen treatment during the last two years had been unsuccessful. Disturbances of the bladder and rectum were present. The tumor was surgically removed. It weighed 6.5 Kg. and was the size of a man's head. The patient died two days later. Post mortem the tumor was found to have metastasized to internal and

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2. Müller, H.: Ztschr. f. rat. Med. **2**:202, 1858.

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7. Curtis, F., and Lefort, R.: Rev. de chir. **43**:1,1911.

8. Pringle, S.: Lancet **1**:1643, 1907.

9. Pototschnig: Beitr. z. path. Anat. u. z. allg. Path. **65**:356, 1919.

external inguinal nodes and the liver. It was composed of vacuolated polygonal cells closely resembling fat cells, arranged in clumps within a bluish-stained fibrillar stroma.

3. Peters[10]: A 68 year old man with a five year history of a slowly growing tumor was operated on and a tumor the size of a fist was surgically removed. It recurred after one year. Roentgen treatment was then given. Post mortem metastases were found in inguinal nodes bilaterally and in regional nodes along the sacrum. This is the first report on a chordoma in which there was involvement of regional lymph nodes.

4. Lewis[11]: Four cases are reported, 2 with metastases. Metastases developed in the neck of a 22 year old woman with a tumor recurring three years after it had been surgically removed. No complete autopsy was performed. A 30 year old man had symptoms of intestinal obstruction. A colostomy was performed and a sacral tumor was resected. Death occurred one year later. Post mortem nodules were seen scattered over the omentum, the peritoneum, the large intestine and the mesentery. Retroperitoneal and mesenteric lymph nodes as well as the capsule of the liver were involved.

5. Stewart[12]: A 65 year old man had a slowly growing tumor for eight years, which was excised. A recurrence was observed after ten years, and the patient died one year later. Post mortem there was a large mass in the left buttock, and there were tiny nodules in the old scar. Large metastases had formed over the right scapula. Histologic examination showed extensive necrosis, hemorrhage and mucoid degeneration. Characteristic polygonal cells with spongy cytoplasm composed the tumor.

6. Argaud and Lestrade[13]: A 14 month old girl, the youngest patient in which this tumor has ever been observed, showed symptoms of intestinal obstruction. Post mortem a tumor the size of an infant's head was found within the pelvis. Metastases were found in the liver. Bone lesions, possibly of osteomalacic nature, were considered as a questionable causal factor.

7. Podlaha and Pavlica[6]: A 44 year old peasant woman complained of intermittent pain in the sacral region. A tumor (570 Gm.) was removed, and three weeks later roentgen therapy was started. Six months after operation there was recurrence of the growth, with accompanying pains. The patient died after the disease had run a course of one and a half years. Post mortem tumor cells were found in lymphatics and veins within the gluteal muscles. No metastases were seen in distant organs.

8. Conway[14]: A 20 year old woman complained of progressive constipation. Three years later, following the diagnosis of inoperable carcinoma of the rectum, colostomy was performed. Two months after operation examination revealed a growth within the rectal lumen, discharging mucus and pus. Three weeks later enlargement of a cervical gland was noted. Biopsy established the diagnosis of chordoma. Death occurred three and a half years later, following rapid loss of weight. Post mortem a large mass was found surrounding the rectum and fixed to the coccyx, involving the perirectal tissues, the peritoneum, the mesocolon, the ovaries, the posterior surface of the uterus and both ureters. Nodules were present in the trigone of the bladder, the great omentum, the mesentery, the peritoneum and the surface of the diaphragm. There was also involvement of lymph nodes along the aorta, the inferior vena cava, the external iliac arteries, in the inguinal region, along the stomach, the pancreas, the roots of the lungs and the thoracic region of the spinal column, continuous with an enormous mass along the right carotid artery to the base of the skull.

9. Willis[15]: A 36 year old woman first noticed a diffuse swelling in the upper part of the left thigh. Three years later difficulty in micturition and radiat-

Fig. 1.—External appearance of the tumor in the back.

10. Peters, W.: Deutsche Ztschr. f. Chir. **151**:191, 1919.

11. Lewis, N. D. C.: Arch. Int. Med. **28**:434, 1921.

12. Stewart, M. J.: J. Path. & Bact. **25**:40, 1922.

13. Argaud, R., and Lestrade, A.: J. A. M. A. **86**: 1731, 1926.

14. Conway, C. A. G.: Mag. London (Roy. Free Hosp.) School Med. for Women **26**:7, 1929.

15. Willis, R. A.: J. Path. & Bact. **33**:1035, 1930.

ing pain developed. The tumor was diagnosed first as sarcoma, and a roentgenogram revealed numerous exostoses. The patient died at the age of 41. Postmortem examination disclosed a sacral tumor diagnosed as chordoma continuous with the tumor in the thigh. There were metastases in both lungs, the heart, the liver, the spleen, both kidneys, the thyroid gland and the skin of the neck. A possible relationship between the numerous exostoses and the tumor on the basis of a constitutional tendency toward skeletal anomalies was considered.

10. Willis[10]: At necropsy a middle-aged man showed a rounded mass in the right buttock occupying the true pelvis, with destruction of the lower part of the sacrum, and continuous with an ulcerated postsacral lesion. Extreme bilateral suppurative pyelonephritis and cystitis had developed. Three metastatic nodules were found in the liver. Typical physaliferous cells are described. There was an abundance of extracellular mucin.

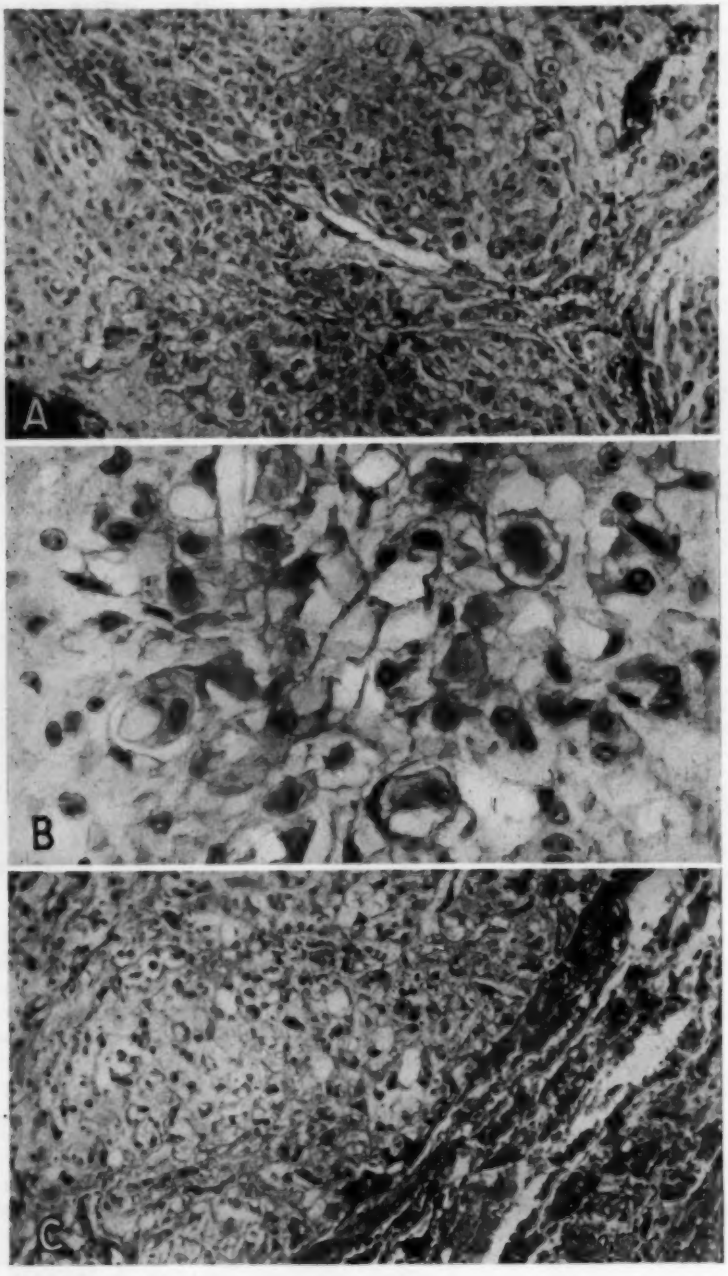

Fig. 2.—*A*, primary tumor in sacrococcygeal region; × 110.
B, higher power magnification of the primary tumor with a typical physaliferous cell; × 460.
C, metastatic nodule in the liver; × 110.

16. Willis, R. A.: Australian & New Zealand J Surg. **3**:92, 1933.

The usual duration of these cases was several years. The symptoms were chiefly rectal and urinary. No definite relationship to age or sex could be found. Trauma has repeatedly been suggested as a causative agent for the growth of these tumors. It is quite possible, however, that the tumors were already present at the time of injury and were merely incited to more vigorous growth by the trauma.

The following case is reported because of two interesting and extraordinary features: first, the enormous size of the tumor and, second, its spread to distant organs.

A 58 year old Russian physician who had always been in excellent health complained of gnawing pain in the sacroiliac region, for which he was unsuccessfully treated. Pruritus ani, urinary frequency, dysuria and progressing constipation soon developed. Rectal examination detected on the posterior wall of the rectum a rounded protuberance corresponding to a subcutaneous mass beneath the skin in the sacral region. A roentgenogram revealed areas of decreased density in the third, fourth and fifth sacral segments and the coccyx. Biopsy of the tumor established the diagnosis of chordoma. On that occasion a large bilateral mass was found over the sacrococcygeal region intruding into the rectal ampulla and infiltrating the gluteus muscles. The lesion was considered too extensive for removal, and the patient was treated with roentgen rays by Dr. Maurice Lenz in the Presbyterian Hospital and later at Montefiore Hospital. This caused a definite decrease in the size of the tumor. But catheterization continued to be necessary. The bowels moved in response to daily enemas. The patient was in excellent general health, with normal temperature, pulse rate and blood pressure. There was no loss of weight and no general weakness. Roentgen treatments were repeatedly given with consequent regression of the tumor. During the intervals between treatments, however, the mass increased greatly in size. After one year, nodular prominences appeared in the region of the left inguinal nodes; by biopsy the swellings were identified as chordoma. Roentgenograms at that time showed complete absence of the cortex of the fifth sacral segment, whereas in previous ones a thin cortical zone could still be traced.

Two years later a lump appeared in the skin of the right leg; the right inguinal nodes were also involved, and the mass in the sacrococcygeal region had grown extensively around the hips and was bleeding easily. In July 1943, five years from the onset of symptoms, the patient was brought to the Presbyterian Hospital following a fall down some steps which fractured several ribs and caused profuse bleeding from the tumor in the back. Despite infusions of dextrose and saline solutions and various stimulants, he died the next day.

Postmortem Examination: Over the entire lower part of the back, encircling the loins and hips up to the anterior abdominal surface, huge cauliflower-like nodular masses were present, forming a broad band 30 cm. in width which ended anteriorly about 3 cm. from the midline. Most of the skin above the tumor appeared intact, but in several areas necrosis and hemorrhage had occurred, disrupting the skin and emitting a repulsive odor. The tumor when removed from the body together with the covering skin and underlying infiltrated subcutaneous tissues weighed approximately 9 Kg. (fig. 1).

On the cut surface it had a glassy, jelly-like, white, translucent appearance. A nodule similar in structure, measuring 4 by 3 cm., was present in the skin of the right leg, and another, 1 cm. in diameter, was on the medial aspect of the left thigh. Both inguinal regions contained many prominent white translucent nodules. The scrotum was considerably thickened and edematous. The sacrum showed marked destruction of all vertebral bodies except for a small cortical zone on the right side. The vertebral bodies were transformed into whitish friable material containing remnants of softened bone and hemorrhagic marrow spaces. They were surrounded and partly infiltrated by white gelatinous tumor tissue. At the site of the promontorium there was a deep cavity due to complete necrosis of the corresponding vertebral body. It was lined by red and yellow friable material. Tumor was found adjacent to the bladder and the rectum without invasion of these organs. It infiltrated the pelvic connective tissue, involving the retroperitoneal and periaortic lymph nodes in this region.

At both pulmonary bases were several small gelatinous white nodules embedded in dense retracted scar tissue. The liver presented two round nodules similar in structure to the tumor in the back. Two such nodules, 2 and 3 cm. in diameter, were found in the right parietal pleura posteriorly. The fractured ribs (seventh, eighth and ninth on the left) revealed no evidence of tumor infiltration. There were no other essential pathologic changes.

Histologic Examination: The tumor was composed of an edematous loose-meshed stroma in which groups or rows of cells were seen. The latter were mostly polygonal and varied greatly in size, some of them being unusually large. The cytoplasm was pale and eosinophilic and often contained a single large or several small vacuoles. The nuclei were quite irregular, deeply stained or more vesicular, occasionally rod shaped and eccentric within the vacuolated cells. Multinucleated cells were numerous. Mitoses were not frequently encountered. Bluish-staining granular material was found around the tumor cells. Larger groups of cells with their delicate stroma were surrounded by dense connective tissue strands so that the tumor appeared divided into separate lobules. Dilated and congested capillaries with quite conspicuous endothelial lining cells were running through the stroma, and occasional areas of diffuse hemorrhage were observed. Tumor cells could not be traced within veins or lymphatics. The typical "physaliferous cells" were seen in many sections (fig. 2).

In Mayer's mucicarmine stain occasional tumor cells took a faint reddish stain.

A sudan IV stain revealed many small or single large fat droplets within tumor cells.

A Foot-Bielschowsky stain showed no definite uniform reticulum; only occasional single tumor cells were surrounded by delicate reticulum fibers. Glycogen often described as being present within the tumor cells could not be demonstrated since alcohol-fixed tissue was not preserved.

All metastatic nodules had a similar histologic appearance.

The spinal cord showed secondary degeneration and gliosis within the columns of Goll, probably caused by compressing tumor masses.

SUMMARY

An unusually large sacrococcygeal tumor diagnosed as chordoma metastasized to the regional and inguinal lymph nodes, the liver, the lungs, the pleura and the skin of the right leg and thigh. Ten cases of chordoma with metastases have been described in the literature.

HISTOPLASMOSIS

Report of Its Occurrence in a Dog

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Histoplasmosis, or "reticulo-endothelial cytomycosis," [1] is a disease produced by a yeastlike organism, Histoplasma capsulatum of Darling,[2] belonging to the imperfect fungi. Since 1926, when the first case was discovered in the continental United States by Watson and Riley,[3] a total of about 40 additional cases have been recorded. About one fourth of the patients were infants of 18 months or less of age; the others were distributed in age groups up to 69 years. The mortality has been almost 100 per cent. At present no treatment is available. The mode of transmission of the organism is likewise unknown.

The first case in which the diagnosis was made during the life of the patient was reported by Dodd and Tompkins [4] in 1934; the patient was an infant 6 months old. The organisms were observed in the peripheral blood. H. capsulatum was first cultivated from autopsy material by De Monbreun.[5] He proved experimentally that it was the etiologic factor of this disease. He later, in 1939, isolated the organism from the liver of a live dog and announced that "a natural host of this disease other than man has been found."

The discovery that the dog, a household pet, may serve as a natural host brings up the question whether or not this animal might play a role in the transmission of this disease to man either directly or indirectly.

REPORT OF A CASE

A foxhound, 4 years old, weighing 50 pounds (22.5 Kg.), was brought to a local veterinarian [6] for disposal. The dog was weak and listless and looked to be about 12 years old. All superficial lymph nodes were greatly enlarged, especially the axillary and the subinguinal groups, which interfered with his movements. It was thought that the dog had Hodgkin's disease.

The blood count made prior to a lethal dose of soluble pentobarbital U. S. P. showed: red blood cells, 4,140,000; leukocytes, 22,700. The differential count was: neutrophils, 47 per cent; lymphocytes, 37.5 per cent; monocytes, 15 per cent; eosinophils, 1.5 per cent. About 7 per cent of the lymphocytes included in the aforementioned figure appeared intermediate to the monocytes and might have been counted among the latter.

At necropsy the following observations were pertinent:

The most striking was the great enlargement of all lymph nodes: cervical, axillary, subinguinal, anterior mediastinal and retroperitoneal, some measuring 3 by 7 cm. They were discrete, and their cut surfaces were pale pink and somewhat bulging. The spleen was approximately twice the normal size and firm. Its cut surfaces were dark red with scattered diffuse nodules here and there. The liver was light brown-red and somewhat enlarged. The cut surface appeared greasy, with occasional dark mottled areas. The lungs seemed of the usual color and texture. Their cut surfaces revealed slight protruding bronchi. Small nodular areas of consolidation were also seen. No changes were noted in the rest of the viscera, and therefore no material was saved for microscopic study.

Tissues were fixed in Helly's fluid. Sections were stained either with Mayer's acid-hemalum and eosin or with a new stannic–alizarin blue triple stain.[7]

The erythropoietic elements of the bone marrow were markedly diminished, and fat cells were almost completely absent. The predominant cells were lymphoid and plasma cells with a relatively small number of myelocytes and stab forms. A few organisms were seen in what appeared to be phagocytosing reticular cells. The usual pattern of the lymph node was almost completely obliterated by strikingly monotonous cell aggregates consisting of lymphoid cells and reticular elements. Many mitotic figures were seen, as well as macrophages containing organisms, singly and in groups. Large collections of macrophages with organisms, in various stages of hyalinization, were visible in regions corresponding to medullary sinusoids. The subcapsular sinusoids were completely obliterated in the larger nodes, though in sections from smaller nodes an occasional one still remained visible. Here and there solid cords of cells appeared in the subcapsular sinusoids. These had large vesicular nuclei, deeply stained, with scarcely discernible cytoplasm. In a few places the cords extended into capsular vessels, filling the latter more or less completely. There was no evidence of fibrosis, necrosis or hemorrhage. The tissues of the capsule were unequally infiltrated with lymphoid cells. In the spleen the capsule and the trabeculae were approximately of the usual thickness. Nodular masses of lymphoid cells were encountered between the capsule and its peritoneal covering. There were only moderately infiltrated areas in the capsule proper. The splenic nodules when present had indefinite margins. The entire pattern of the organ was permeated diffusely with cells strikingly similar to those observed in the lymph nodes. The splenic sinusoids were almost completely obliterated. Multinuclear cells with deeply stained nuclei and pink cytoplasm were

From the Department of Histology and Embryology, University of Oklahoma School of Medicine.

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3. Watson, C. J., and Riley, W. A.: Arch. Path. **1**:662, 1926.

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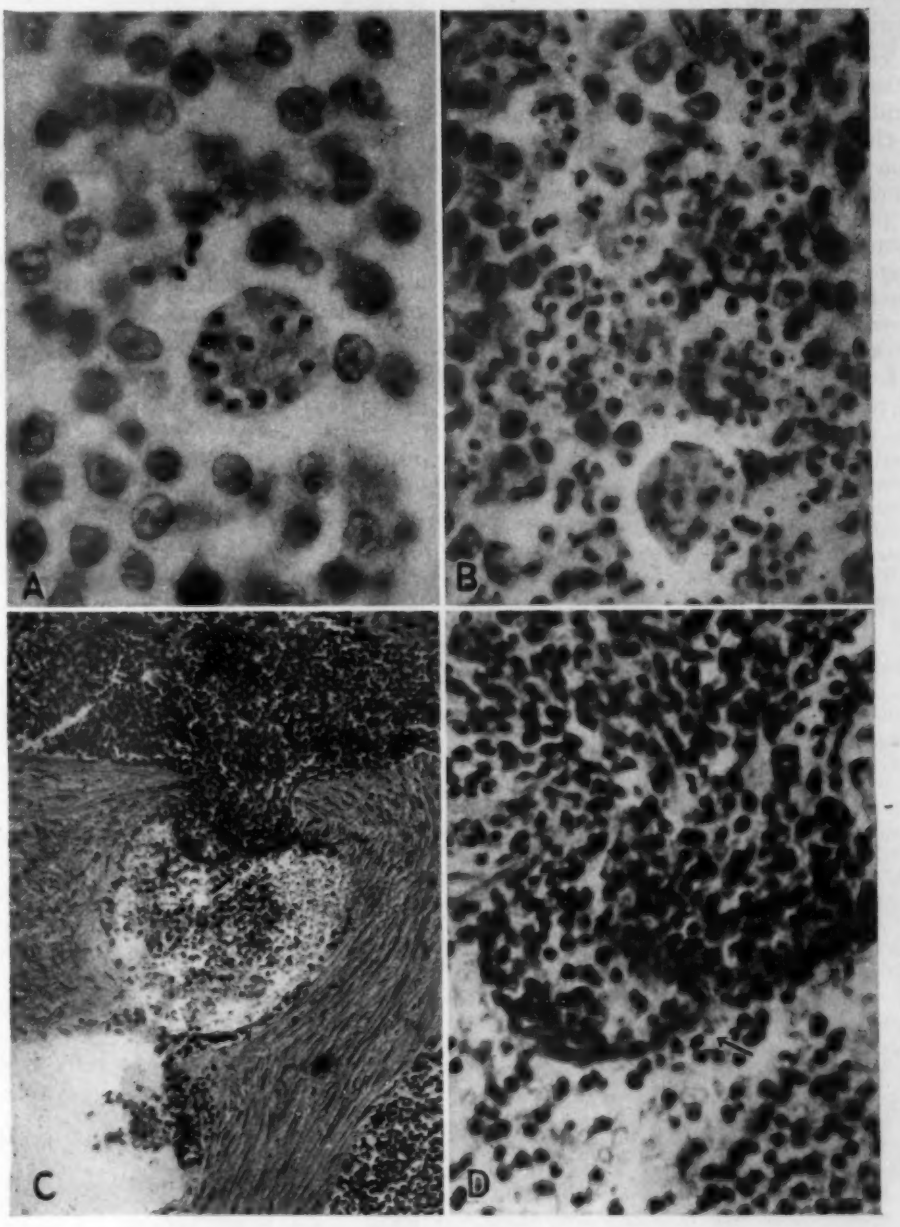
5. De Monbreun, W. A.: (*a*) Am. J. Trop. Med. **14**:93, 1934; (*b*) **19**:565, 1939.

6. Dr. H. W. Schmees, of the Northeast Veterinary Hospital, gave me permission to use this material.

7. Thuringer, J. M.: Stain Technol., to be published.

numerous. Some of these resembled fused macrophages, while others appeared to be megakaryocytes. Islands of blood-forming tissues were present here and there. Free organisms were seen everywhere, and macrophages containing organisms were scattered singly or in groups. The hyperplastic cells were especially abundant along the trabeculae. In several locations the trabeculae were invaded, and the infiltrating cells formed subendothelial intrusions. In a few places they appeared to break into the lumen of a vein. In the liver the arrangement of the liver cell cords into lobules was barely discernible. The intralobular sinusoids were compressed except in a few lobules with distended centrolobular veins, in which the sinusoids appeared distended for a short distance. In these sites the liver cells appeared granular, while elsewhere they were vesciular, with eccentrically placed nuclei and irregular cell outlines. They did not appear as signet rings. The Kupffer cells were inconspicuous. A few of them contained organisms. In the portal areas there was some slight infiltration consisting of lymphocytes, plasma cells and large mononuclear cells. The sublobular veins were most involved in this perivascular



A, cells in a lymph vessel. Note the macrophage with Histoplasma capsulatum, free organisms and a "lymphoid" cell. × 1,000. *B*, section of a lymph node showing macrophages with intracellular organisms. Budding may be observed in many of the free organisms. × 750. *C*, section of spleen with a trabecular vein. A hyperplastic mass of cells apparently is forcing a way through smooth muscle by some cytolytic process. A break in the endothelial lining may be seen near the arrow. × 125. *D*, same field as *C*, showing details of the break in the endothelium. The arrow points to several free organisms. "Lymphoid" cells are seen pouring into the vein. × 500.

infiltration. In the lungs there was a variable amount of lymphoid and plasma cell infiltration in the bronchial and bronchiolar mucosa and along the bronchial arteries. Occasionally nodules of lymphoid cells appeared in the interstitial tissue, and in a few areas the alveoli were replaced by solid tissue aggregates resembling tubercles. Occasional multinuclear cells, free organisms and some hyalinization were seen.

COMMENT

In this case the lesions were apparently confined to the lymphatic apparatus and consisted of a generalized hyperplasia of the reticular and lymphoid elements. It was possible to observe a progression of hyperplastic cells from the distended lymph nodes by way of capsular vessels. In some places they were filled with closely packed lymphoid cells only, while elsewhere many phagocytes with organisms, extracellular organisms, lymphoid cells and hyalinized debris were seen within the vessels.

In the spleen the hyperplastic cell masses appeared to exert a destructive action on the trabeculae, which in the dog consist largely of smooth muscle. It appeared that the cell masses produced a cytolytic effect on the latter. Subendothelial nodules of cells were found. These probably corresponded to the subendothelial tumors observed by Agress and Gray [8] in the pulmonary arteries. Here, however, it was observed that these masses frequently broke through into the lumen of the vessel, the cells becoming liberated into the blood stream. Trabeculae with arteries were also invaded, but the hyperplastic cells, after reaching the musculature of the artery proper, began to spread, forming a sheath around the latter which preceded the secondary invasion of the arterial wall proper.

SUMMARY

A second case of canine histoplasmosis calls attention to the possibility that the dog may serve as the natural host and transmitter of this disease to man.

8. Agress, H., and Gray, S. H.: Am. J. Dis. Child. **57**:573, 1939.

MIXED TUMOR OF THE LIP

Report of Two Cases, Including One of Mixed Tumor of the Lower Lip

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Since Ahlbom[1] published his comprehensive monograph "Mucous and Salivary Gland Tumors" in 1935 there have been an increasing number of reports of so-called mixed tumors of the salivary glands in the literature. These have been reported in a variety of locations, of which the parotid and submaxillary glands are the commonest. The relative rarity of mixed tumor of the upper or the lower lip makes it particularly worthy of consideration. It is recognized that mixed tumor of the upper lip occurs far more frequently than that of the lower lip.

In 1938 Eggers [2] in a review of the literature collected 64 cases of mixed tumor of the lip, including 4 in which the site was the lower lip reported by Bruggemann,[3] Pupovac,[4] Wood [5] and Huguier.[6] Later in the same year Lepera and Catalano [7] collected from the literature 75 cases of mixed tumor of the lip, including 3 in which the lower lip was the site reported by Franssen,[8] Wood [5] and Huguier.[6]

Eggers [2] stated that he had included only cases accessible to him, and it is apparent that some of the cases cited by Lepera and Catalano [7] are not incorporated in his series.

After personally checking the references cited by both authors, to eliminate duplications, we find: 1. The literature contains 4 verified cases of mixed tumor of the lower lip, reported by Bruggemann,[3] Pupovac,[4] Wood [5] and Franssen.[8] (We have not included the case of Huguier [6] since the diagnosis was based on a clinical report without pathologic confirmation.) 2. There are 74 cases of mixed tumor of the upper lip reported up to 1938, including 1 case by Eggers [2] and 1 case by Lepera and Catalano.[7]

Since the reviews cited, 7 additional cases of mixed tumor of the upper lip have been reported: 1 by Rabenalt,[9] 1 by Giardina,[10] 4 by Cid [11] and 1 by Warshaw.[12] There have been no additional reports of mixed tumor of the lower lip since that of Franssen [8] in 1934.

Recently 2 cases of mixed tumor of the lip, in one of which the lower lip was involved, were encountered here in the course of routine examination of surgical specimens submitted to the department of pathology for diagnosis.

CASE 1 (FIG. 1)

A Negro woman aged 24 years had noticed a small hard painless swelling in the left side of the lower lip for six years. It had not progressed in size. There was no history of trauma. The only discomfort was that caused by the slight protrusion of the lower lip.

A firm tumor mass measuring 10 mm. in diameter was embedded in the substance of the left side of the lower lip midway between the angle and the midline. The tumor was movable in the tissues but firmly adherent to the outer skin. It was enclosed in a fibrous tissue capsule, which made removal easy, an incision being made along the interior line of the lower lip.

The specimen was a firm, encapsulated, slightly lobulated tumor mass measuring 10 by 6 by 5 mm. The cut surface was grayish white, opaque and smooth. Histologic study revealed solid sheets, thin strands and tubular structures of epithelial cells in a fibromyxomatous stroma with areas of dense hyaline cartilaginous tissue scattered throughout. The epithelial cells varied from spindle to cuboidal in shape, with scant, rather deeply staining cytoplasm and hyperchromatic nuclei. No mitotic figures were seen. There was no evidence of calcification or of the presence of stratified squamous epithelium. The tumor was enclosed in a well developed fibrous connective tissue capsule.

The pathologic diagnosis was noncancerous mixed tumor, salivary gland type, of the left side of the lower lip.

CASE 2 (FIG. 2)

A white woman aged 36 had had a small hard nodule immediately beneath the skin, just to the right of the midline of the upper lip for two years. The nodule was a little less than 10 mm. in diameter. It was very firm, and the skin over it was extremely thin and moderately adherent. She stated that the nodule had become quite dark within the past week. This was difficult to explain since there was no history of trauma or of treatment of any kind. She had received roentgen therapy about six or eight months before, which apparently had little or no effect on the size of the nodule. A small elliptic incision was made extending from just above the nodule to just below the nodule and including the thinnest portion of the skin over the summit. When the nodule was exposed in position, it was dark and was rather adherent to the surrounding tissues. It was dissected and completely removed, but the dissection was tedious. After the tumor had been removed the dark color completely disappeared.

The specimen was an encapsulated firm tumor mass measuring 5 by 5 by 3 mm. The cut surface was white,

From the Department of Pathology, Medical College of Virginia.

1. Ahlbom, H. E.: Acta radiol., 1935, supp. 23, p. 1.
2. Eggers, H. E.: Arch. Path. **26**:348, 1938.
3. Bruggemann, A.: Ztschr. f. Krebsforsch. **9**:345, 1910.
4. Pupovac, D.: Deutsche Ztschr. f. Chir. **49**:77, 1898.
5. Wood, F. C.: Ann. Surg. **39**:57 and 207, 1904.
6. Huguier: Bull. Soc. de chir. de Paris **5**:437, 1852.
7. Lepera, L., and Catalano, F. E.: Semana méd. **2**:682, 1938.
8. Franssen, R.: Ann. d'anat. path. **11**:275, 1934.
9. Rabenalt, H.: Zentralbl. f. Chir. **65**:2647, 1938.
10. Giardina, G.: Tumori **12**:455, 1938.
11. Cid, J. M.: An. de cir. **5**:252, 1939.
12. Warshaw, D.: M. Rec. **154**:87, 1941.

opaque and smooth. Histologic examination showed a varied appearance. There were masses of epithelial cells arranged in tubules and long tapering anastomosing strands. The stroma was fibromyxomatous tissue mingled with areas of hyaline cartilage. The epithelial cells varied in shape from spindle to cuboidal, the cytoplasm was scant and the nuclei were hyperchromatic. No mitotic figures were seen. No stratified squamous epithelium or evidence of calcification was present. The pathologic diagnosis was noncancerous mixed tumor of the right side of the upper lip, salivary gland type.

Unlike mixed tumors in other locations, that of the lip is almost universally considered noncancerous. The specimens described were rather uniform in type. Clinically they were slow growing, small, movable, well circumscribed and nonulcerating. Histologically they presented the usual complex structure of mixed tumor of the salivary gland. They were well encapsulated and there was no evidence that they were infiltrating the capsule or the surrounding tissues. Both were regarded clinically and pathologically as noncancerous.

Fig. 1 (case 1).—Mixed tumor of the lower lip showing the fibrous tissue capsule, the epithelial strands and the acini interspersed in the fibromyxomatous stroma with prominent areas of cartilaginous tissue. Hematoxylin-eosin; × 180.

There is still much discussion concerning the origin of mixed tumors of the salivary glands. The multiple theories concerning their histogenesis are attributable to their marked histologic variability. Ahlbom [1] gave a historical review of these tumors and discussed the various theories regarding their nature and development. Of these, that of endothelial origin has largely been abandoned.

Prevailing theories are that they arise from (1) salivary gland epithelium, (2) branchial cleft tissue or (3) embryologically misplaced tissue. From extensive studies Krompecher [13] concluded that these tumors were wholly epithelial in origin. In 1933 Zymbal [14] cultured tissue from tumors of salivary glands and demonstrated the development of mucoid and cartilaginous tissue by metaplasia from glandular epithelium. Ahlbom [1] and Ewing [15] agreed that many of these tumors may be wholly of epithelial origin but that not all can be explained on this basis. Harvey, Dawson and Innes [16] and Sheldon [17] called attention to the

13. Krompecher, E.: Beitr. z. path. Anat. u. z. allg. Path. **44**:51, 1908.

14. Zymbal, W. E.: Beitr. z. path. Anat. u. z. allg. Path. **91**:113, 1933.

15. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, chap. 26, pp. 790-798.

(Footnotes continued on next page)

presence of basket cells in the normal salivary gland and also in many of the mixed tumors. These cells were studied extensively by Zimmermann [18] and their ectodermal origin is generally accepted.

Cohnheim [19] in 1882 suggested that mixed tumors could be derived from embryonal branchial anlages. The branchiogenic theory can explain satisfactorily the origin and the marked variation of the histologic structure of some of those tumors attached to the salivary glands. tissues of the extremities is strong evidence against branchial cleft origin.

Hempelmann and Womack,[21] among others, discussed the concept of mixed tumors developing from multipotential cells in which a varying degree of differentiation has occurred as a result of a disturbance in tissue environment in early embryonic life. The degree of differentiation which the tumor tissue shows would depend on the age of the embryo when the disturbance occurred. Experimental evidence shows that a

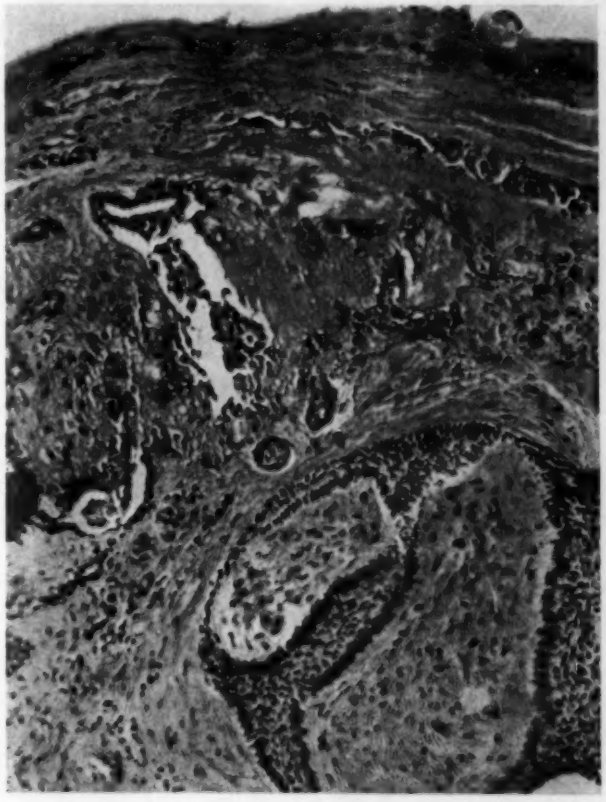

Fig. 2 (case 2).—Mixed tumor of the upper lip showing the fibrous tissue capsule and the epithelial strands interspersed in a fibromyxomatous stroma. Hematoxylin-eosin; × 180.

McFarland [20] presented extensive justification for this concept. However, the frequent occurrence of mixed tumors in the lips, the palate, the lacrimal glands and even the skin and subcutaneous disturbance in tissue environment in early embryonic life leads to the development of the monster and the teratoma, but so far there has been no attempt to apply this theory to mixed tumors.

Cuneo and Veau [22] expounded the theory of the origin of mixed tumors from embryonically misplaced tissues. Swinton and Warren [23] and Eggers [2] agreed that the frequent occurrence of

16. Harvey, W. F.; Dawson, E. K., and Innes, J. R. M.: Debatable Tumours in Human and Animal Pathology, London, Oliver & Boyd, Ltd., 1940, chap. 3, p. 17.

17. Sheldon, W. H.: Arch. Path. **35**:1, 1943.

18. Zimmermann, K. W., in Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 5, pt. 1, p. 130.

19. Cohnheim, J.: Allgemeine Pathologie, Berlin, August Hirschwald, 1882.

20. McFarland, J.: Surg., Gynec. & Obst. **63**:457, 1938.

21. Hempelmann, L. H., Jr., and Womack, N. A.: Ann. Surg. **116**:34, 1942.

22. Cuneo, R., and Veau, V.: Cong. internat. de med. chir. **10**:278, 1900.

23. Swinton, N. W., and Warren, S.: Surg., Gynec. & Obst. **67**:424, 1938.

mixed tumors at the sites of fusion and foldings in the complex embryonic development of the face is favorable evidence in support of this concept. The disparity in frequency between mixed tumor of the upper lip and that of the lower lip can also be explained on this basis since the embryologic development of the upper lip is more complex.

SUMMARY

Two mixed tumors of salivary gland type are reported, including one in the lower lip. Both of these tumors were well encapsulated, contained epithelial cell nests and cords and myxomatous and cartilaginous tissue and were considered noncancerous clinically and on histologic examination.

In the literature, including the cases reported here, there are now 82 reported cases of mixed tumor of the upper lip and only 5 verified cases of mixed tumor of the lower lip. In all the reported cases the tumor was considered noncancerous. It has been nine years since an instance of mixed tumor of the lower lip was reported, the last having been that reported by Franssen [8] in 1934.

Forensic Medicine

FULMINATING MENINGOCOCCIC INFECTION WITH BILATERAL MASSIVE ADRENAL HEMORRHAGE (THE WATERHOUSE-FRIDERICHSEN SYNDROME)

WITH SPECIAL REFERENCE TO THE PATHOLOGY, THE MEDICOLEGAL ASPECTS AND THE INCIDENCE IN ADULTS

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Since 1901 a clinical entity has been recognized by a few clinicians, especially pediatricians, in which children, usually between 1 and 9 years of age, while in apparent health are suddenly stricken with headache, high fever, vomiting and restlessness. A peculiar variegated extreme cyanosis rapidly develops which often resembles postmortem lividity, and this is accompanied by petechiae over the face, the trunk and the extremities. These petechiae rapidly become larger and may form a macular purpura-like rash; finally they become so confluent as to impart a blotchy appearance to the skin. Numerous petechiae are often also present in the conjunctivas. Cardiorespiratory collapse, delirium, stupor, convulsions and coma soon follow, and death occurs within eight to twenty-four hours after onset. As Herrick [1] has stated, "No other infection so quickly slays." Since 1918 this condition has been known as the Waterhouse-Friderichsen syndrome. Most authorities have felt that in the majority of cases it is of meningococcic origin, but to illustrate the confusion which has existed as to the micro-organisms responsible for this syndrome, as recently as 1942 Karsner [2] stated "The condition is apparently due to fulminant bacterial infections; hemolytic streptococci, pneumococci, influenza bacilli, meningococci, and others have been recovered from different cases."

The characteristic changes noted at autopsy in such cases are usually: marked cyanosis; petechial, purpuric and ecchymotic lesions of the skin; conjunctival petechiae; petechiae over the visceral peritoneum and pericardium, and, most diagnostic, the presence of bilateral massive adrenal hemorrhages, with the absence of gross meningitis in the majority of cases.

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1. Herrick, W. W., cited by Boyd, W.: A Textbook of Pathology, ed. 4, Philadelphia, Lea & Febiger, 1943, p. 857.

2. Karsner, H. T.: Human Pathology, ed. 6, Philadelphia, J. B. Lippincott Company, 1942, p. 704.

This absence of marked meningeal involvement supports the observation of Herrick,[3] made during the first world war, that most meningococcic infections are bacteremias before there is metastatic localization in the leptomeninges. This concept has been upheld by the recent great success in reducing the mortality of epidemic cerebrospinal meningitis by early injection of sodium sulfadiazine.

SUPPOSED RARITY OF THE WATERHOUSE-FRIDERICHSEN SYNDROME

Until 1943 only 107 cases of this syndrome had been recorded in the literature, and about 90 per cent of the patients had been infants and children under 9 years of age. Only a few had been adults.

SYNDROME LONG RECOGNIZED BY THE MEDICAL EXAMINER AS THAT OF FULMINATING MENINGOCOCCEMIA

In the experience of my colleagues and myself who as medical examiners investigate violent, sudden and suspicious deaths, this disease complex (cyanosis, purpura, petechiae and bilateral massive hemorrhages in the adrenal glands without gross meningitis) has for many years been recognized as a cause of sudden, unexplained and argumentative deaths not only in infants and children, but also in adults. The experience of the New York medical examiners has been similar.[4]

During the last thirteen years investigators in this office have studied and made autopsies in 19 cases of this disease in the performance of over 10,000 autopsies. Ten of the subjects were infants and children (the majority below 5 years of age) and 9 were adults. Nine of the 19 cases occurred in 1943, most of them in March, April and May, at which time meningococcic infections were prevalent in army camps and among the civil population of New Jersey. Because of this experience with the Waterhouse-Friderichsen syndrome in adults I had predicted that a few

3. Herrick, W. W.: J. A. M. A. **71**:612, 1918.

4. Personal communications to the author.

cases of fulminating meningococcic septicemia would be found whenever cerebrospinal meningitis was prevalent or epidemic and that in view of the pathologic observations even an early diagnosis and the heroic use of the sulfonamide compounds, saline solution, adrenal cortex extract and other modalities could not prevent a fatal issue.

Furthermore, I had asked some of the officers of the Medical Corps of the United States Army to watch for this type of meningococcic infection, should they be in contact with epidemic cerebrospinal meningitis. The occurrence of such cases offers an excellent opportunity to settle some of the controversial and confusing aspects of this syndrome.

Diagnosis Easily Made at the Autopsy Table.—The gross changes observed at autopsy in cases of this syndrome are so typical that the office of the Chief Medical Examiner of Essex County has for many years made positive diagnoses of "fulminating meningococcal septicemia sine meningitis (Waterhouse-Friderichsen syndrome)" without waiting for bacteriologic confirmation. Health authorities have been immediately notified, inspectors sent out and contacts examined, and in our opinion some lives have been saved by action on these direct, immediate results of autopsies.

The purely anatomic diagnosis is further supported by the fact that in a large experience with all types of infections due to streptococci, staphylococci, pneumococci and other organisms this office has never found bilateral *massive* hemorrhages in the adrenal glands in any of these infections or in any condition other than fulminating meningococcemia.

It must be admitted, however, that small areas of necrosis and hemorrhage, usually microscopic in size, may sometimes be found in the various septicemias. We have had no experience with smallpox, diphtheria, scarlet fever, typhus and other contagious diseases in which purpura and petechiae may occur.

Massive hemorrhage in the adrenal glands, often bilateral, may also occur in the newborn infant following birth injury and after vitamin deficiency, and it is seen in "blunt force compression injuries" to the abdomen and lower part of the chest. Such lesions, of course, can never be mistaken for those of the Waterhouse-Friderichsen syndrome.

MEDICOLEGAL ASPECTS OF THE WATERHOUSE-FRIDERICHSEN SYNDROME

The literature contains little or no reference to the medicolegal problems which may arise as sequelae of this disease. Since death in this condition is so rapid (usually at less than twenty-four hours after onset of symptoms), it is often reported to the Medical Examiner as a sudden unexplained death. Many patients are found dead without medical attention or die shortly after admission to hospitals. Other deaths from this cause have to be investigated because of the suspicion of food or chemical poisoning, foul play or negligence.

A short résumé of the 19 cases reported in this series will best demonstrate the medicolegal aspects of this disease as it is encountered in civil life.

1. Adult found dead in bed. No medical history except that subject was well when last seen alive.

2. Adult died in hospital twenty hours after vomiting mushrooms. Mushroom poisoning suspected.

3. Infant found dead in crib. Two other children taken to hospital for suspected scarlet fever. Meningococcic meningitis developed in one and subacute meningococcic sepsis in the other without purpura or meningitis. Both recovered.

4. Infant dead on arrival at hospital; had been sick eight hours. No diagnosis.

5. Adult died two minutes after admission to hospital and forty-eight hours after onset of gastrointestinal symptoms. Relatives wanted to sue a "fish market."

6. Child died at home eight hours after onset of vomiting. Father requested an autopsy, blaming spoiled food and the attending physician for death.

7. Child died in hospital twenty-four hours after onset. Six weeks previously it was knocked off bicycle and spleen ruptured. Splenectomy with uneventful recovery. Parents considered accident as cause of death.

8. Infant died in arms of mother; had wakened in night with fever and vomiting; sick only eight hours. Intelligent parents insisted on autopsy.

9. Five year old child died in hospital twelve hours after onset. Case not reported to Medical Examiner and body released by hospital. Death certificate signed "bronchopneumonia and intestinal convulsions." Two days later father charged that eating of a hot dog and negligence of hospital and attending physician were responsible for death. Undertaker stated that father wanted a police investigation and said child was covered with "blue spots."

10. Infant died in hospital one hour after admission and nine hours after onset. Parents could not understand sudden death although a correct diagnosis was made at hospital.

11. Adult died in hospital one hour after admission. Correct diagnosis made from cyanosis, purpura and conjunctival petechiae. Autopsy made to confirm diagnosis and as public health measure.

12. Adult died in hospital eight hours after admission and twenty-four hours after onset. Worked in defense paint factory and possibility of occupational benzene poisoning was suggested. Relatives claimed that he had been poisoned or had met with foul play but were satisfied after autopsy.

13. Infant died in hospital eight hours after admission and eighteen hours after onset. Brought to hospital in moribund condition by parents. Parents requested autopsy.

14. Child died in hospital five hours after admission. Condition correctly diagnosed from cyanosis, purpura, petechiae and rapid collapse. Autopsy made to confirm diagnosis and as public health measure.

15. Child died in hospital seventeen hours after onset. Diagnosis of Waterhouse-Friderichsen syndrome made by interns after witnessing previous autopsies on persons with this condition. Autopsy made to confirm diagnosis and as a public health measure.

16. Adult found dead in bed. Police suspected "mugging" or manual strangulation. Subject dead for two days and body in bad state of decomposition. Subject well when last seen alive.

17. Adult died in hospital nine hours after onset. Police stated that husband often "beat her up" and disappeared after learning she had died. Correct diagnosis made at hospital. Autopsy to confirm diagnosis and release husband from suspicion.

18. Adult taken sick on automobile bus. Died three hours after admission to hospital. Body covered with macular purpuric rash. Correct diagnosis made at hospital. Autopsy made to confirm diagnosis and to forestall any civil action.

Nineteen Cases of the Waterhouse-Friderichsen Syndrome Investigated by the Medical Examiner's Office of Essex County (Newark), N. J.

Case	Month	Year	Male	Female	White	Negro	Child	Adult
1	Dec.	1930	..	+	+	..	.....	26 yr.
2	April	1935	..	+	+	..	.....	16 yr.
3	April	1936	+	..	..	+	3 yr.	.....
4	April	1936	+	..	..	+	19 mo.	.....
5	April	1936	..	+	+	..	.....	26 yr.
6	Sept.	1941	..	+	+	..	4 yr.	.....
7	Oct.	1941	+	..	+	..	13 yr.	.....
8	Dec.	1942	..	+	+	..	1 yr.	.....
9	Feb.	1943	+	..	+	..	5 yr.	.....
10	March	1943	+	..	+	..	11 mo.	.....
11	March	1943	+	..	..	+	.....	31 yr.
12	April	1943	+	..	+	..	.....	32 yr.
13	April	1943	..	+	..	+	14 mo.	.....
14	April	1943	+	..	..	+	10 yr.	.....
15	May	1943	+	..	+	..	7 yr.	.....
16	May	1943	..	+	..	+	.....	36 yr.
17	May	1943	..	+	..	+	.....	39 yr.
18	Aug.	1943	..	+	+	..	.....	30 yr.
19	Aug.	1943	+	..	+	..	.....	50 yr.
Total.....			10	9	12	7	10	9

19. Adult found dead at foot of stairs of a "flop house." No medical history. Autopsy made to determine cause of death.

ANALYSIS OF NINETEEN CASES OF WATERHOUSE-FRIDERICHSEN SYNDROME

A statistical summary of the 19 cases investigated by the office of the Medical Examiner of Essex County during the last thirteen years is shown in the table.

Season.—Most of the cases listed in table 1 occurred in March, April and May, the season in which meningococcic infections are most prevalent in this part of the country.

Sex.—In this series the distribution of the cases between the sexes is about equal.

Race.—Twelve of the patients were white persons and 7 were Negroes. Since the Negroes of this area comprise about 12 per cent of the population, these figures would indicate a higher incidence among Negroes. This fact might be explained by their crowded living conditions.

Age.—Ten of the 19 patients in this series were infants and children, the youngest being 11 months and the oldest 13 years of age. Seven of the 10 patients were under 6 years of age. This age incidence is in agreement with that for most of the reported cases of the Waterhouse-Friderichsen syndrome. On the other hand, 9 patients were adults of the following ages: 16, 26, 26, 30, 31, 32, 36, 39 and 50 years. This indicates a relatively high incidence in adults in comparison with most of the series recorded in the literature.

Duration of Life.—Most of the 19 patients died within twenty-four to thirty-six hours after onset of symptoms. This is in accord with most published experience, as it is usually stated that death occurs within forty-eight hours after onset.

AVAILABLE CLINICAL DATA IN THE NINETEEN CASES

No medical history or clinical data could be obtained in 5 cases. In the remaining 14 cases the clinical and laboratory data were often meager because of the short duration of the disease. Briefly, the following symptoms were noted:

Onset.—Headache was complained of in only 6 cases. However, since many of those afflicted were infants, the stated incidence of this subjective symptom is of little value. Vomiting was mentioned in 9 cases and gastrointestinal symptoms in 5 cases, with considerable abdominal pain in 1. Chills were recorded in 4 cases and fever in 12 cases (often reaching 103 to 106 F.). Pains in muscles of the neck and over the body were noted in 5 cases. Cervical rigidity was noticed in only 5 cases. Neurologic findings were too scant to be of value.

Skin and Conjunctiva.—Marked cyanosis was observed in 11 cases. One patient called attention to the unusual blue color of his skin. Purpuric and ecchymotic spots varying in size from petechiae to large areas, often confluent and forming a maculopurpura-like rash, were seen in 9 cases. Conjunctival petechiae, often numerous, were noted during life in only 7 cases but were seen at autopsy in many more cases. These lesions of the skin may develop so quickly that they sometimes appear while the patient is being examined.

Rapid Progress of Symptoms.—In a few hours after onset the condition of the patients became rapidly worse, and soon they were moribund. Delirium, stupor and coma were recorded in 10 cases. Convulsions were noted in 8 patients, and these were frequently adults. Cardiorespir-

atory collapse, which occurred in all cases, was noted only in 9. The pulse was often imperceptible, but in some instances the rate reached 200 per minute, with a respiratory rate frequently of 60. Blood pressure readings in 5 cases showed systolic pressures between 50 and 60, with diastolic readings unobtainable.

Laboratory Findings.—In but 7 cases were good laboratory data available. Leukocytosis was recorded in 7 instances, the highest leukocyte count being 99,500, with 99 per cent polymorphonuclear neutrophils. In 3 other cases the leukocyte counts were 20,200, 20,600 and 43,000. Positive blood cultures were obtained in 6 cases; all revealed meningococci. Spinal fluid was examined in 6 cases and in all was sterile and showed no leukocytes. Examination of petechiae in 2 cases showed gram-negative diplococci, later identified as meningococci. No chemical examination of blood was recorded in any case.

Diagnosis. — The Waterhouse-Friderichsen syndrome was correctly diagnosed in 7 persons and was suspected after death in 3 by interns who had previously witnessed autopsies on these persons.

Failure to Save Life.—In 5 cases attempts to overcome the profound meningococcemia by intravenous injections of sodium sulfadiazine and dextrose solutions were futile and made no apparent impression on the course of the disease. The patients progressed rapidly from the overwhelming meningococcemia into a moribund state. Whether penicillin might save some patients is conjectural, as it has not been used enough in combating meningococcic infections to warrant an opinion as to its efficacy against fulminating meningococcemia.

Heroic efforts to combat the cardiorespiratory collapse on the assumption that the symptoms were due to an acute insufficiency of adrenal cortex failed in 5 cases to resuscitate the moribund patient. The intravenous administration of salt or of adrenal cortex extract, which often brings a patient with Addison's disease out of the crises, had no effect in 5 cases. This suggests that the much more important factor is the overwhelming bacterial invasion which may cause symptoms simulating insufficiency of the adrenal cortex.

The Waterhouse-Friderichsen syndrome is apparently that of a fatal infection with a duration usually of from eight to forty-eight hours from onset of symptoms. It is extremely doubtful if any patient has recovered. This could never be proved, since meningococcemia occurs with lesions of the skin and cardiorespiratory collapse identical with that in the Waterhouse-Friderichsen syndrome and at autopsy no significant lesions of the adrenal glands are shown. Many of the patients with the latter syndrome recover.

PATHOLOGIC DATA IN THE NINETEEN CASES

Autopsies were made in all 19 cases.[5]

External Examination of Body.—Variegated, often extreme cyanosis and lividity over the face, the chest and the dependent portions of the body were outstanding in 16 cases. This lividity faded on pressure.

Purpuric, ecchymotic and petechial lesions of the skin were often innumerable and were found in all but 1 case. These lesions were scattered over the head, the chest, the abdomen and the extremities often down to the finger tips and the feet. They ranged in size from those just visible and petechial in character to larger, often confluent areas several centimeters in size. None of these lesions faded on pressure.

Conjunctival petechiae were present in 14 cases. These were more numerous in the conjunctivas of the lower lids but also might be seen in the ocular conjunctivas in many cases. They were scarlet red, had no pale, raised or depressed centers such as are seen in various forms of endocarditis and could not be distinguished from similar petechiae occurring in death from various types of asphyxia.

Internal Examination of Body.—Petechial or larger hemorrhages were seen over the peritoneum, especially the serosa of the small intestine and mesentery (9 cases), and occasionally also over the parietal peritoneum. Similar hemorrhages were found in the epicardium over the posterior surface of the heart in 8 cases. All these locations are frequent sites for asphyxial hemorrhages, from which the petechiae must be differentiated. Pleural petechiae were seen in 7 cases, usually over the visceral pleura.

Bilateral massive hemorrhages in the adrenal glands were found in all 19 cases, and this constitutes the diagnostic finding in this type of fulminating meningococcemia.

Chromaffin structures other than the adrenal medulla were examined in 2 cases, especially the carotid bodies and the semilunar ganglions, but neither gross nor microscopic lesions were found. The pituitary gland was examined in 2 cases, and no microscopic lesions were found.

The brain showed no gross evidence of meningitis in 15 cases. In many cases there was evidence of increased intracranial pressure as indi-

5. In 2 cases which were reported to the Medical Examiner and released, permission for autopsy was obtained. Dr. Ellis L. Smith, medical director of the Essex County Isolation Hospital, gave permission for the inclusion of these cases.

cated by the congestion of the leptomeninges and the flattening of the convolutions over the surface of the brain. The gray matter of the cortex and of the basal nuclei was often "rose colored," denoting active hyperemia or encephalitis.

In 3 cases there was a small amount of visible exudate in the leptomeninges. In 1 case there was definite purulent exudate over the base of the brain, and smears showed numerous gram-negative diplococci. In those cases in which there was suggestive or definite evidence of meningitis the duration of the disease was over thirty-six hours.

The deep cervical lymph nodes were extremely red and hyperemic in the 3 cases in which they were examined, suggesting the importance of the upper respiratory tract as the portal of entry of the infection.

The mesenteric lymph nodes, Peyer's patches and the solitary lymphoid tissue of the intestine were not hyperemic or congested in any of the cases. It should be recalled that these structures are sometimes actively inflamed in poliomyelitis, suggesting the intestinal tract as a portal of entry.

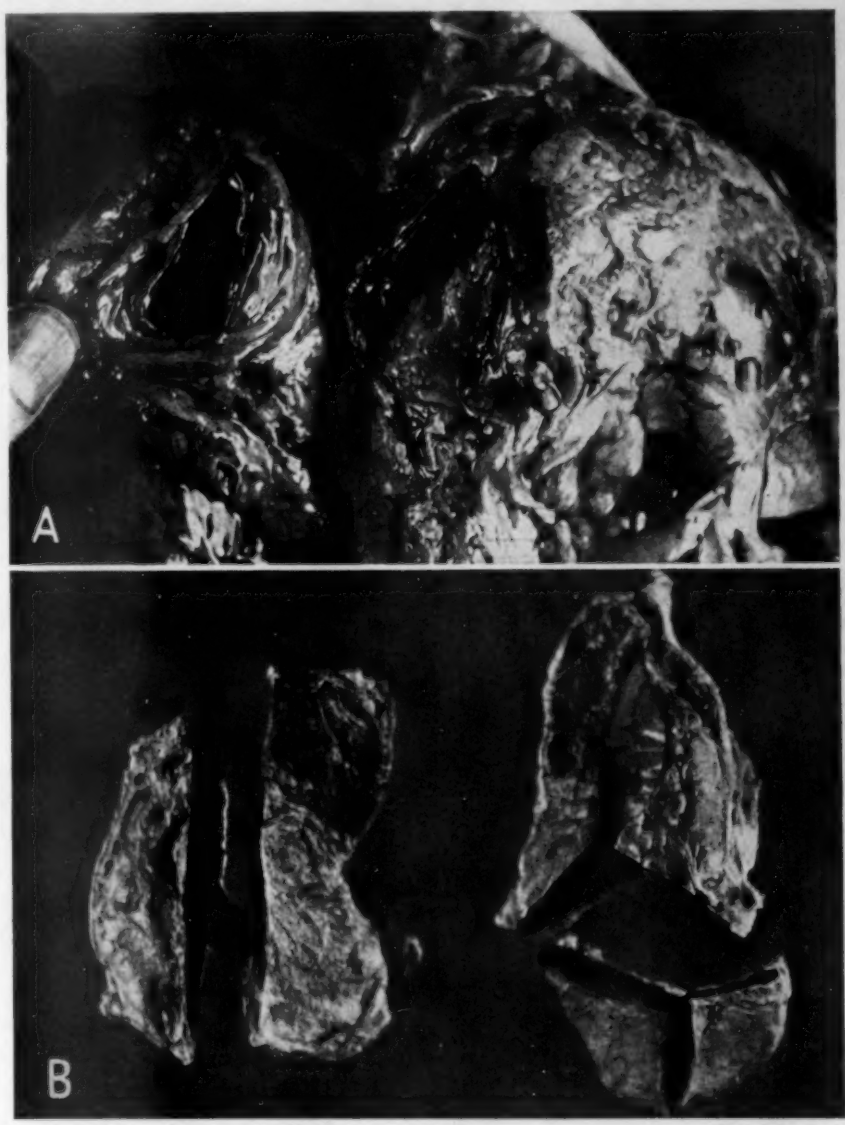

Fig. 1.—Adrenal glands from adults dying of fulminating meningococcemia (Waterhouse-Friderichsen syndrome). *A*, glands seen through the parietal peritoneum (case 11). *B*, glands dissected out for weighing and measurement (case 18). The left gland weighed 13 Gm. and measured 6 by 4 by 1 cm. The right gland weighed 11 Gm. and measured 5.5 by 3.5 by 0.8 cm. The normal adult adrenal gland weighs 5 to 6 Gm. and measures 4 by 2.5 by 0.5 cm.

Aside from the hemorrhages in the adrenal glands and the occasional early involvement of the meninges the viscera showed no significant pathologic lesions with the exception of acute cloudy swelling of the myocardium, the kidneys and the liver and terminal pulmonary edema.

Status lymphaticus of questionable degree was found in only 3 of the 19 cases. The thymus in these cases was large, thick and fleshy, but the lymphoid tissue in the spleen and the intestinal tract was not excessive, and the mesenteric lymph nodes were not hyperplastic.

tures of petechiae in 2 cases showed a growth of meningococci. Smears and cultures from the leptomeninges showed meningococci in only 3 cases.

Postmortem Chemical Examination of the Blood.—No chemical analyses of the blood were recorded in any of the 19 cases during life nor does the literature give sufficient information on chemical alterations observed in the blood which

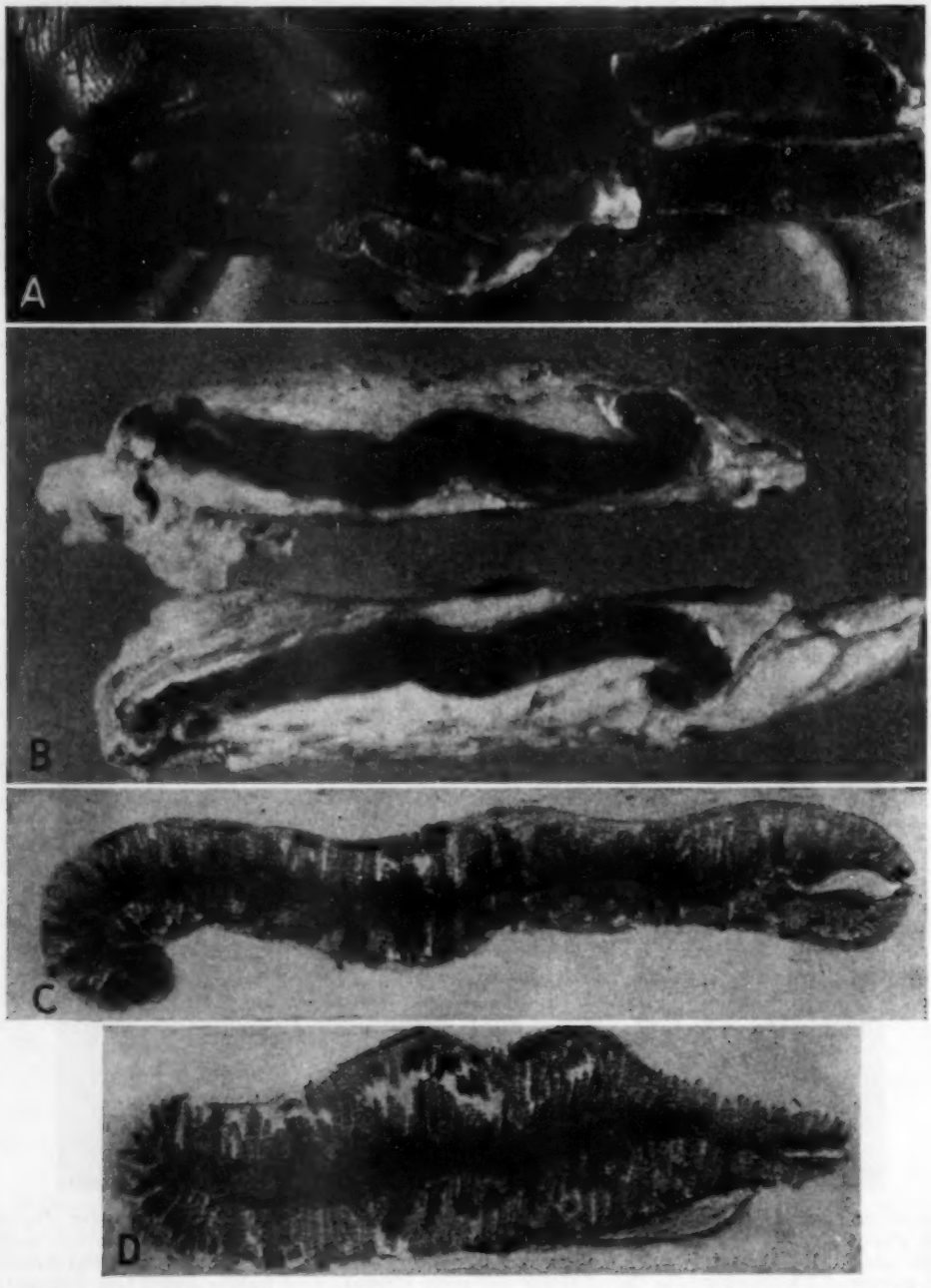

Fig. 2.—Adrenal glands from 4 cases of fulminating meningococcemia (Waterhouse-Friderichsen syndrome) to illustrate the localization of the hemorrhagic areas in the cortex. *A* and *B*, photographs of two bisected adrenal glands; *C* and *D*, drawings of two adrenal glands. In all these glands the streaky, or parallel, distribution of the hemorrhagic areas in the middle and inner zones of the cortex may be noted. Practically no medullary substance is visible in any of the sections.

Postmortem Bacteriologic Confirmation.—Cultures of blood taken from the right side of the heart in 6 cases revealed meningococci. Cul-

would indicate an insufficiency of adrenal cortex in this condition.

Blood taken at autopsy of 1 adult six hours after death showed:

Blood sugar ..... 104 mg. (normal, 80 to 120)
Blood chlorides ... 513 mg. (normal, 450 to 500)
Blood cholesterol.. 120 mg. (normal by Bloor's method, 140 to 180).

This one examination would indicate that there is no hypoglycemia and no lowering in blood chlorides such as has been found in the acute insufficiency of adrenal cortex in Addison's disease. On the other hand, the blood cholesterol was definitely low. Since the adrenal glands are important depots for storage of cholesterol and are frequently pale and washed out in many forms of acute and chronic sepsis, it is suggested that a low level of blood cholesterol may be connected with the cardiovascular collapse.

Furthermore, there is no absolute proof that an insufficiency of adrenal cortex is present—in many cases a considerable portion of the cortex is often intact, and in the aforementioned case the pertinent chemical determination of the blood would not support this contention. There is unfortunately almost a complete lack of data on the chemical constituents of the blood in the Waterhouse-Friderichsen syndrome. Perhaps, if the patients lived long enough, some changes in the sodium ion pattern might occur.

PATHOLOGY OF THE HEMORRHAGES IN THE ADRENAL GLANDS

Gross Appearance.—As soon as the renal regions are exposed at autopsy, one is startled by the remarkable appearance of both adrenal glands as seen through the overlying peritoneum. Both glands are deep purplish red. They retain their normal shape but are definitely swollen and tense in their capsules and are increased in size and weight (fig. 1).

On section, each adrenal gland appears to be the seat of massive hemorrhage which is destroying almost the entire gland. Usually a thin outer shell or fringe may be seen, denoting that the outer layer of the cortex is best preserved.

The hemorrhagic areas are deep red and will not wipe off. We have never encountered the large blood cysts described by some authors (such an appearance, however, might be seen if there is rapid postmortem decomposition or if the autopsy is made a considerable time after death).

Appearance Under Hand Lens.—When a cut section is made through the entire thickness of the adrenal gland and examined with a hand lens (preferably after fixation) it will be seen that in the majority of cases the most extensive damage is in the middle and inner layers of the cortex, even though the entire gland seems to be destroyed.

In the middle zone (zona fasciculata) the hemorrhagic areas have a definite pattern—innumerable parallel red streaks or rays extend throughout most of the zone, often reaching the capsule in irregular areas (figs. 2 and 3).

In the inner zone (zona reticularis) the hemorrhages often have a lacelike or reticulated appearance.

Through confluency of these areas, large hemorrhagic extravasations occur which obliterate all markings. In many instances these large hemorrhages extend into the central portions of the gland, surrounding the central vein, and have given the impression that the lesions originate in the medulla. It should be recalled, however, that in many adrenal glands true medullary substance is scant, with many sections showing little or no medulla. In such areas the inner layer of the cortex is in almost direct contact with the opposing cortex, only a small amount of loose connective tissue intervening. In addition the zona reticularis is often mistaken for the medulla. The medulla is not essential to life.

Microscopic Examination.—Study reveals that what appeared to the naked eye to be streaky and reticulated areas of hemorrhage in the middle and inner zones of the cortex is really enormous capillary distention. The straight, parallel capillaries of the middle zone are stuffed with red blood cells with little or no surrounding inflammatory reaction and no evidence of thrombosis or embolism (fig. 4).

This capillary distention in the inner zone (zona reticularis) and sometimes in the outer zone (zona glomerulosa) may assume an appearance of a reticulated network conforming with the normal course of the capillaries in these two zones (fig. 5).

The enormous distention of the capillary system of the cortex plus the toxic effect of an overwhelming meningococcemia on the capillary endothelium causes a rupture of the capillary walls and extensive extravasations of blood in the parenchyma of the cortex and often in the central, or medullary, portions of the gland (fig. 6).

When there is extensive hemorrhage in the central portions, it is conceivable that the hemorrhage may act as an obstruction causing still greater capillary distention of the cortex.

In most cases the outer zone (zona glomerulosa) escapes except where it is involved in isolated, irregular areas.

The capsule is not involved in the lesion, and the surrounding tissues rarely show hemorrhage.

In some adrenal glands, especially those from persons who lived a sufficient length of time, small areas of focal necrosis with leukocytic infiltrations may be seen, and in properly stained sections innumerable meningococci may be found scattered in irregular clumps throughout most of the cortical parenchyma.

Aside from these areas of necrosis, the lesion in the adrenal gland is an intense capillary distention of the cortex with large hemorrhagic extravasations in the cortex and central portions of the gland and almost complete absence of any other inflammatory reaction.

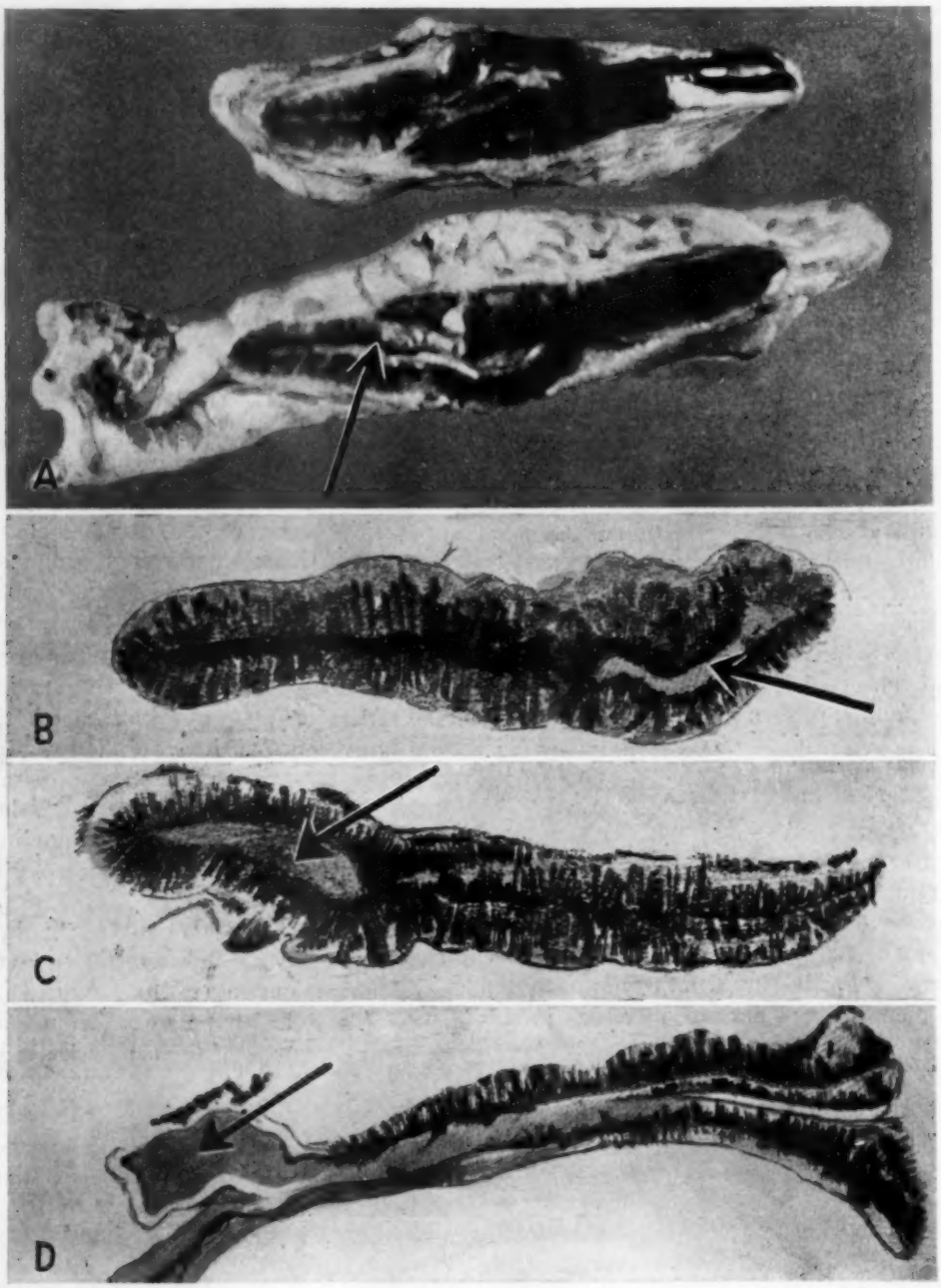

Fig. 3.—Adrenal glands from 4 cases of fulminating meningococcemia (Waterhouse-Friderichsen syndrome) showing that there is little or no involvement of the medullary portions. *A*, photograph of a bisected adrenal gland; *B*, *C* and *D*, drawings made from three other adrenal glands. Note that in all these glands the visible medulla (marked by arrows) is not involved in the hemorrhagic process.

VARIOUS EXPLANATIONS OF THE LESIONS OF THE GLANDS

Various theories have been proposed to explain the selective action on the adrenal glands

in this disease. A few of the theories are herewith discussed:

Embryologic Interpretation.—Since the nervous system, the skin and the adrenal medulla have a common origin from ectoderm and these organs are the main ones affected in meningococcic infections, it has been intimated that this might explain the hemorrhages in the adrenal glands.

Our series shows that in most cases the location of the hemorrhages is in the cortex, which is mesodermal in origin. Other mesodermal structures are also attacked in meningococcic infections—for instance, the endocardium and the joints in some subacute and chronic forms of meningococcemia.

High Incidence of Status Lymphaticus.—Numerous authors have felt that the occurrence of the lesions of status lymphaticus in many cases of the Waterhouse-Friderichsen syndrome was more than coincidental.

As most of these observers were reporting the disease in infants, in whom various degrees of lymphoid hyperplasia are common, there is a reasonable explanation for such opinions.

Since there is some experimental evidence to support a relationship between the cortex of the adrenal gland, lymphoid hyperplasia, insufficiency of adrenal cortex and status lymphaticus, the latter condition has assumed some importance in explaining the location of the lesions in these glands.

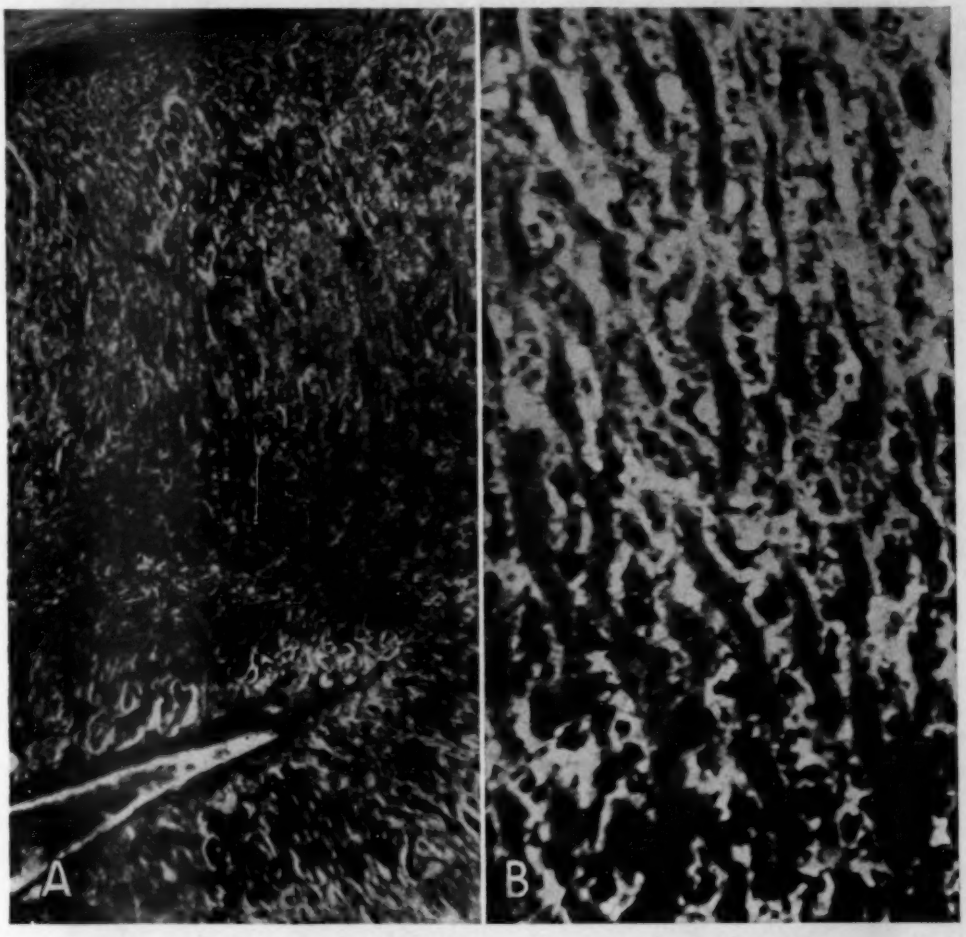

Fig. 4.—*A*, low power photomicrograph showing the capillary distention in the middle zone. Such capillary engorgement has been mistaken for actual hemorrhage. *B*, high power photomicrograph showing the same lesion. The straight, parallel overdistended capillaries of the middle zone (zona fasciculata) may be noted.

This series indicates that there is no actual connection between the occurrence of the status lymphaticus constitution and this type of meningococcemia. This view is taken in spite of the fact that I am one of the few pathologists who still strongly upholds the belief in the existence of persons of the thymicolymphatic status and in the importance of this pathologic picture in interpreting many forms of sudden death, especially those observed in infants.

Venous Thrombosis.—Thrombosis of the central veins has been given as the cause of the

hemorrhages in the adrenal glands. Such occlusions could easily explain the capillary distention in the cortex on a purely obstructive basis. However, we have seen no evidence of such thrombosis in this series.

Bacterial Embolism.—Many workers hold that the cutaneous lesions in this disease are embolic phenomena; therefore, it was natural to assume that the lesions of the adrenal glands might be due to mechanical blocking of the capillaries by bacteria.

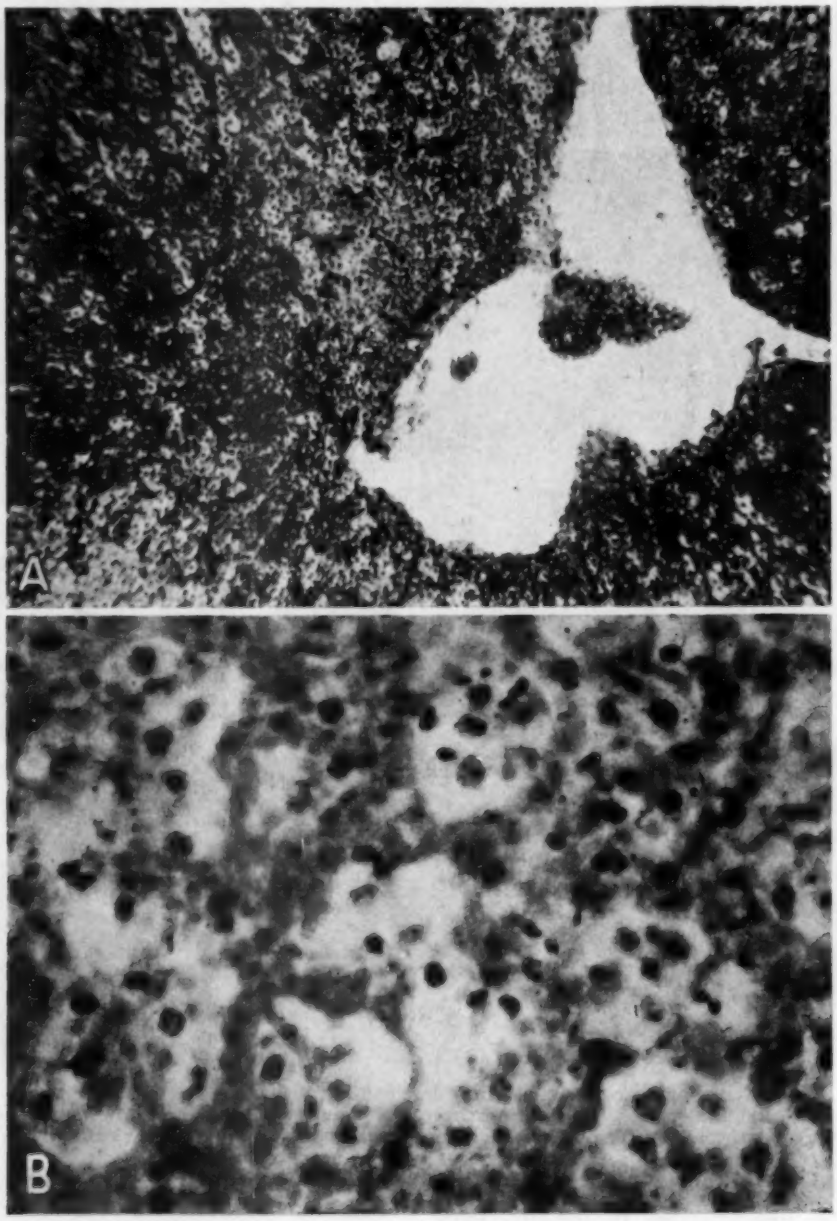

Fig. 5.—*A*, low power photomicrograph showing the congestion of the inner zone (zona reticularis). The reticulated appearance of the overdistended capillaries may be noted in the upper left hand corner. *B*, high power photomicrograph of the same tissue, showing the reticulated course of the congested capillaries.

Arterial Thrombosis.—There is likewise no indication of arterial blockage in this series. It would be difficult to conceive of symmetric bilateral arterial thrombosis of the adrenal glands, and the pathologic process would be more in the nature of an ischemic infarction of the gland.

However, even in the most pronounced forms of bacteremia it is difficult to picture mechanical blocking of even the smallest capillaries by microorganisms alone. For instance, in sepsis following abortion such mechanical blockage frequently occurs, but here the organisms are clumped

together along with agglutinated leukocytes, blood platelets and other elements and form embolic masses of appreciable size.

We have seen no evidence in our cases of actual mechanical blocking of capillaries by meningococci alone, although innumerable diplococci may be found in the surrounding parenchyma.

It would appear that the best explanation of the hemorrhages in the adrenal glands would be the increased permeability of the capillaries of the cortex, which is anatomically so rich in capillaries. No other organ with the exception of the skin has such an extensive delicate capillary bed. The adrenal gland receives its arterial blood directly from its capsule; the blood flows through

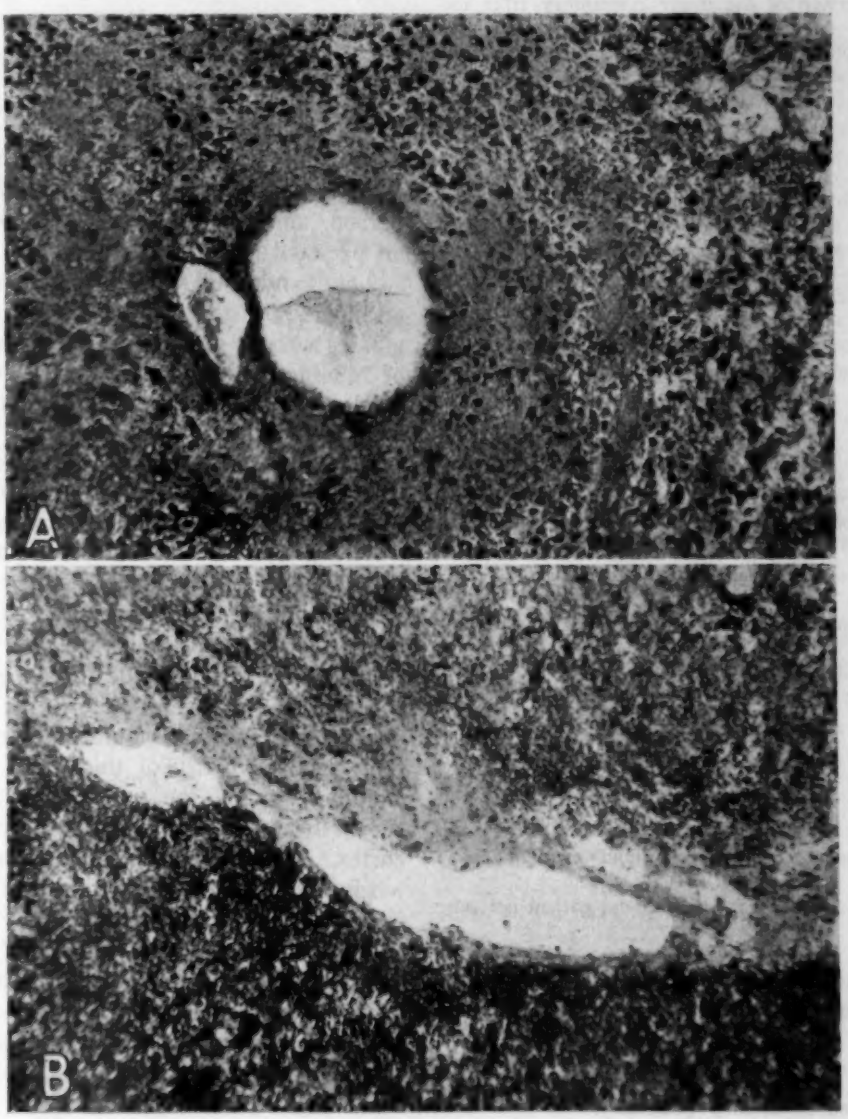

Fig. 6.—*A* and *B*, low power photomicrographs showing extensive hemorrhage in the central portions of the adrenal gland around the central veins.

Effect of Meningococcic Toxins.—The most reasonable theory which has been offered to explain the hemorrhages in the skin and in the adrenal glands in this disease is that the toxins liberated during overwhelming meningococcemia have a direct toxic effect on capillary endothelium and cause rupture of the walls with resulting hemorrhagic extravasation.

delicate capillaries, which seem to have little or no wall in places so that the blood is brought into intimate contact with the cells of the gland, after which the blood flows directly into the central veins.

GREAT VARIABILITY IN MENINGOCOCCIC INFECTIONS

In a study of meningococcic infections one is impressed by the great variability in the clinical

and pathologic types. However, they are usually clearcut. The main types of meningococcic infection are briefly tabulated here.

I. Meningococcemia with Meningitis
1. Epidemic cerebrospinal meningitis (cerebrospinal fever, spotted fever, meningococcic meningitis)
(*a*) Over 75 per cent of all meningococcic infections are of this type.
(*b*) An infection of the upper respiratory tract followed by bacteremia with metastatic localization in the leptomeninges rather than direct extension through the ethmoid labyrinth.
(*c*) Suppurative meningitis the main feature.
(*d*) Epidemic and sporadic cases, with or without rashes.
(*e*) During epidemics cases of fulminating disease occur with purpura, petechiae and maculopapular rashes. Cardiorespiratory collapse may suggest insufficiency of adrenal cortex, and some recoveries have been attributed to use of adrenal cortex extract. However, significant adrenal lesions are not found at autopsy.
(*f*) Early diagnosis and intravenous injections of sulfadiazine have reduced the fatality rate from more than 40 to 5 per cent or less.

II. Meningococcemia without meningitis
[Whenever meningococcic meningitis is prevalent, cases of acute, subacute and chronic meningococcemia without meningitis appear. These may form a considerable group in some epidemics, as high as 25 per cent.]
1. Acute meningococcemia without meningitis (common)
(*a*) Fever, malaise, joint pains, rashes and leukocytosis.
(*b*) Early diagnosis and sulfonamide compounds have checked infection.
2. Subacute meningococcemia without meningitis (common)
(*a*) Mild symptoms suggesting rheumatic fever or other infections.
(*b*) Usually there is recovery.
3. Chronic meningococcemia without meningitis (unusual)
(*a*) Low grade fever for long periods; patient not very sick.
(*b*) Usually there is recovery unless complications, such as meningococcic endocarditis, develop.
4. Fulminating, fatal meningococcemia without meningitis
(*a*) Purpura, petechiae, rashes and bilateral massive hemorrhages in the adrenal glands (Waterhouse-Friderichsen syndrome) (unusual).
(*b*) No rashes or other lesions of the skin but presence of massive hemorrhages in the adrenal glands (Waterhouse-Friderichsen syndrome) (rare).
(*c*) Purpura, petechiae and rashes but no massive hemorrhages in the adrenal glands. (Chiefly in children; not uncommon.)

III. Traumatic meningococcic meningitis
1. Fracture of the skull extending through accessory sinuses of head (occasionally seen).
2. Suppuration in the middle ear, the mastoid process and the frontal sinuses (rare).

CONCLUSIONS

The series of 19 cases of the Waterhouse-Friderichsen syndrome analyzed in the preceding report is perhaps the largest observed by one person, and the conclusions are based entirely on personal experience.

This syndrome (cyanosis, purpura, petechiae and bilateral massive hemorrhages in the adrenal glands without gross meningitis) is always that of fulminating, fatal meningococcemia, in which death occurs before there is marked invasion of the leptomeninges.

This condition is not as rare as the literature would suggest and is not limited to infancy or childhood but is likely to be encountered in adults whenever meningococcic infections are prevalent.

Making the clinical and the anatomic diagnosis is comparatively simple after attention has been called to the condition.

The hemorrhages in the adrenal glands apparently are due to several factors: the toxins liberated by the enormous number of meningococci, the selective affinity of this toxin for capillary endothelium and the presence of a susceptible rich delicate capillary network in the adrenal cortex. However, death is due more to the overwhelming meningococcemia than to acute insufficiency of adrenal cortex.

On account of the frequency of sudden and unexpected death, the sporadically occurring Waterhouse-Friderichsen syndrome in civilian life must sometimes be differentiated from food or chemical poisoning, trauma, negligence and even homicide.

Notes and News

Appointments.—P. A. Wheeler, formerly associate professor at Washington University, St. Louis, has been appointed associate professor of pathology in Baylor University, Houston, Texas.

Valy Menkin, formerly assistant professor of pathology at the Harvard Medical School, is now associate in research at the Fearing Laboratory of the Free Hospital for Women, Brookline, Mass.

Society News.—The Association of American Physicians plans a one day session, to be held on Tuesday, May 9, 1944, at the Hotel Claridge, Atlantic City, N. J., "unless unforeseeable adverse circumstances arise."

The Society of American Bacteriologists will hold its annual meeting in New York May 3, 4 and 5, 1944. The headquarters will be in the Hotel Pennsylvania, where the scientific sessions will be held. L. S. McClung, 302 Chemistry Building, Indiana University, Bloomington, Ind., is program chairman.

"Fulbright Professorship of Pathology."—The endowment of a professorship bearing this name at Baylor University, Houston, Texas, is announced. The donor is Mrs. Irene Fulbright.

Study of Fungous Infections at Duke University.—It has been announced that Duke University has received a grant from the American Foundation of Tropical Medicine for the purpose of acting as a diagnostic and registry center for fungous diseases of man.

The service may be outlined as follows: 1. Fungi *already isolated* from patients suspected of having fungous disease will be identified. 2. A pathologic study and registry of biopsy and autopsy materials from patients suspected of having fungous infection will be made. N. B. This is not to conflict with the diagnostic service and registry services maintained by the several branches of the armed forces. 3. A complete set of cultures of pathogenic fungi will be sent on request to any medical school for use in teaching courses in tropical medicine. To guarantee arrival of the fungi in a proper state for study, it is necessary that requests for this material be sent at least one month prior to the time that the cultures will be necessary for demonstration.

By special arrangement serologic tests will be done for certain of the fungous infections and vaccines for intradermal testing and for therapeutic use in certain of the fungous infections will be sent on request.

Specimens for pathologic study should be sent to Dr. Roger D. Baker, Duke Hospital, Durham, N. C. All other requests should go to Dr. D. T. Smith, Duke Hospital, Durham, N. C.

Books Received

ANOPHELES GAMBIAE IN BRAZIL 1930 to 1940. Fred L. Soper and D. Bruce Wilson. Cloth. Pp. 262, with 75 illustrations. New York: The Rockefeller Foundation, 1943.

Anopheles gambiae, an African mosquito and an important vector of malaria, secured a foothold in northeastern Brazil in 1930. No sustained effort was made to eradicate this mosquito from the invaded districts until about a decade later, when it caused a terrific outbreak of malaria, in the course of which 15,000 to 20,000 deaths occurred. The book describes the invasion of Brazil by Anopheles gambiae and the eradication of the insects. By means of paris green (a double salt of copper acetate and copper metaarsenite) and pyrethrum Anopheles gambiae "was beaten back, and finally eradicated from the known infested area in less than two years' time. Observations covering a period of a year and a half, including two rainy seasons, after the suspension of all anti-gambial measures, indicate that the eradication has been complete." The authors took an essential part in the program of eradication, which was financed by the Brazilian government and the Rockefeller Foundation. The volume will be a landmark in the literature on the prevention of disease. It tells calmly in scientific style how a large part of the western hemisphere was freed of the menace of malaria—a most remarkable and instructive achievement in public health work.

MEDICAL PARASITOLOGY AND ZOOLOGY. Ralph Welty Nauss, M.D., Dr.P.H., assistant professor of public health and preventive medicine, Cornell University Medical College. Foreword by John C. Torrey, Ph.D., professor (emeritus) of epidemiology. Pp. 534. Price $6. New York: Paul B. Hoeber, Inc., 1944.

This book is based on the experience of the author in laboratory diagnosis and in teaching medical parasitology and zoology. There are four main topics: protozoa parasitic in man; worms parasitic in man; arthropods and transmission of disease; poisonous and venomous animals. The nature, the modes of causation, the diagnosis, the prevention and the treatment of the human diseases caused by these agents are described clearly and concisely. In the appendix are sections on technical matters, lists of venomous snakes in the western hemisphere, a glossary (classification of animal parasites and arthropods, definitions), a bibliography and an index. In the preface is an instructive epitome of a course in medical parasitology and zoology to second year students in ten sessions of three hours each. The presentation is lucid, compact and well arranged, and the illustrations are neat and helpful. The book is a credit to the publisher. It is welcomed as an important, useful, much needed textbook for medical students, teachers and clinicians.

TEXTBOOK OF HISTOLOGY. (Sixth edition of "Lewis and Stöhr.) J. Lewis Bremer, M.D., Hersey professor of anatomy, Harvard University. Rewritten by Harold L. Weatherford, Ph.D., assistant professor of anatomy, Harvard University. Pp. 723, with 598 illustrations. Price $7. Philadelphia: Blakiston Company, 1944.

There are now available for selection by students recent editions of several excellent textbooks of histology, each one characterized by distinctive features in presentation and emphasis of subject matter. This latest edition of the textbook of histology from the Harvard Medical School Department of Anatomy preserves the spirit and the form of the series which originated in 1906 as a free translation by F. T. Lewis of Philip Stöhr's "Lehrbuch der Histologie," to which were added descriptions of the embryologic origin of the tissues and organs as indicated by the subtitle "Arranged upon an Embryological Basis." Dr. Lewis saw this book through a second edition, following which Dr. Bremer took it over for three editions, his latest in 1936. Now the editorship has passed to Dr. Harold L. Weatherford, who in this sixth edition has adhered to the general plan of his predecessors. The outstanding feature of this textbook is the incorporation of an extraordinary amount of embryologic information, achieved through succinct but clear exposition and well chosen illustrations. This feature enhances the value of the book to medical students for reference and review, particularly in relation to the study of pathology.

Much new material has been introduced to keep up with new advances in histology and to replace that considered to be less desirable. The list of references to literature has been weeded and enlarged, and the inclusion of references to pioneer as well as to the most modern papers will appeal to students with a historical bent.

This edition has 598 illustrations, 302 of them new, as compared with 455 illustrations in the fifth edition. Many of the new illustrations are original; others are borrowed from other sources; all are well chosen to supplement the text and increase the value of the book; they account largely for the increased number of pages (143) in this edition and give the book a pleasing appearance.

Especially improved are the sections on the use of the microscope and on the methods used in the study of cells and tissues.

The chapter on cytology has been rewritten and expanded, and that on the endocrine glands has been much improved and modernized without wandering too far into the intricacies of the endocrine system and the hormones. Other chapters remain almost unchanged. A new section on the shape of cells has been supplied by F. T. Lewis. The former section on the placenta has been replaced with a new article by G. B. Wislocki on human placentation, embodying his recent studies and illustrated by some superb photographs of young human embryos. These two sections, though perhaps transcending the usual bounds of a textbook for medical students, will find approval among advanced students and instructors.

As a whole, the edition has maintained the admirable balance of previous editions and has met worthily the example they have set of continued improvement and of concise clear exposition.

PSYCHOSOMATIC MEDICINE. THE CLINICAL APPLICATION OF PSYCHOPATHOLOGY TO GENERAL MEDICAL PROBLEMS. Edward Weiss, M.D., professor of clinical medicine, Temple University Medical School, and O. Spurgeon English, M.D., professor of psychiatry, Temple University Medical School, Philadelphia. Pp. 687. Price $8. Philadelphia and London: W. B. Saunders, 1943.

REPORT OF THE SECRETARY OF THE SMITHSONIAN INSTITUTION AND FINANCIAL REPORT OF THE EXECUTIVE COMMITTEE OF THE BOARD OF REGENTS, 1943. Pp. 95. Price 25 cents. Washington, D. C.: United States Government Printing Office, 1943.